1 July 2005

Science

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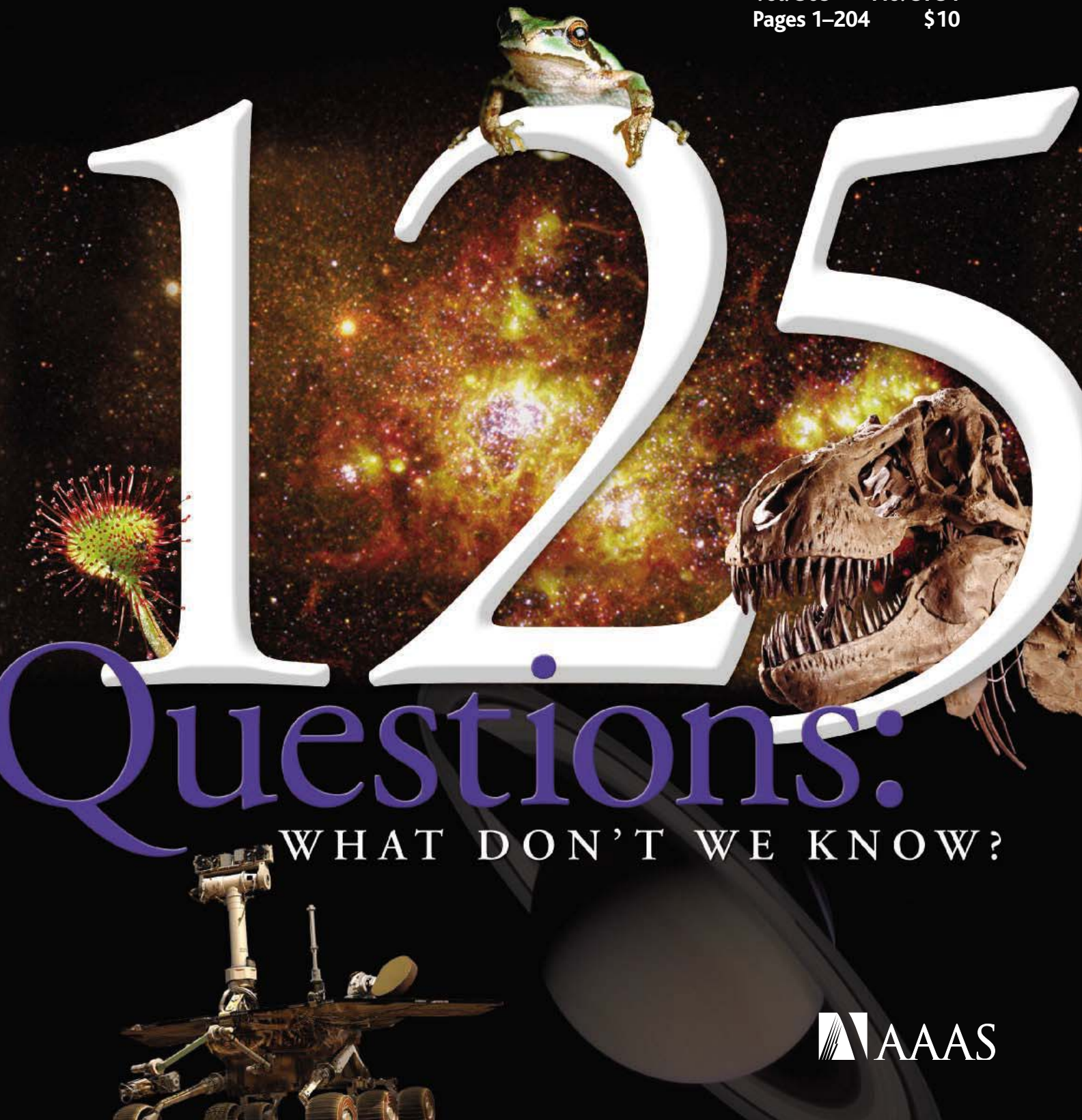

125 Questions:

WHAT DON'T WE KNOW?

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Science

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125TH ANNIVERSARY

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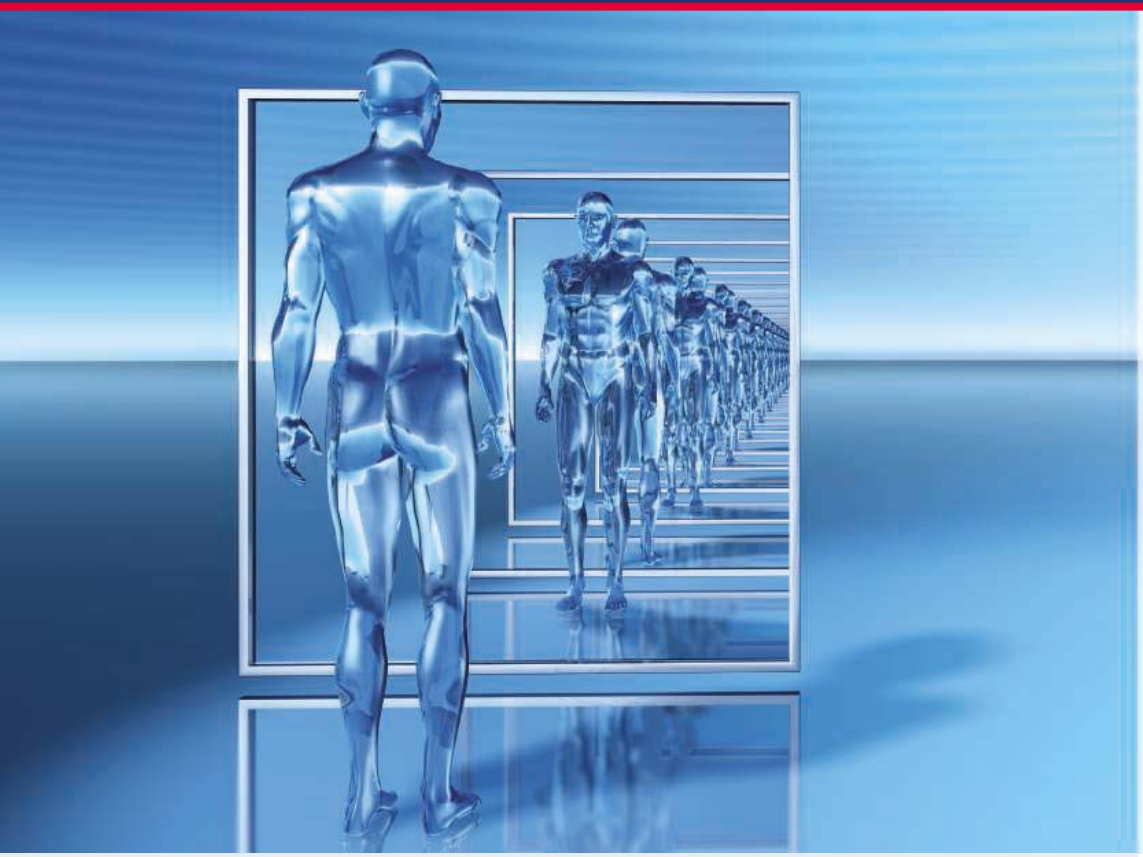

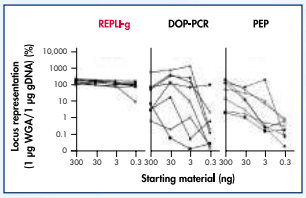

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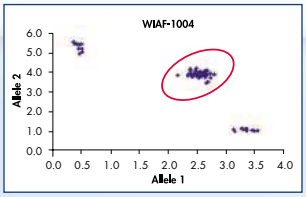

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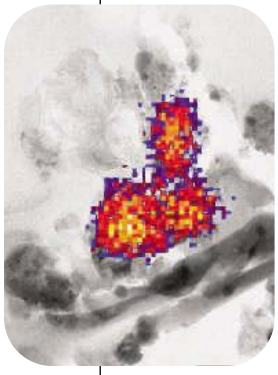

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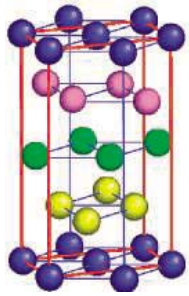

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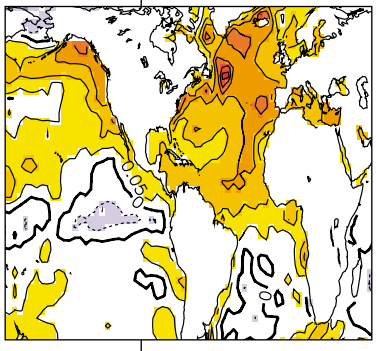

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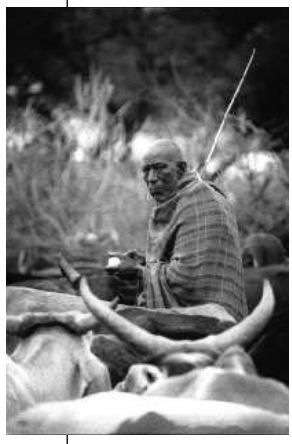

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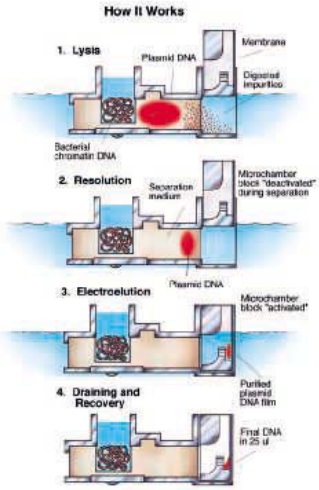

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Pulsar Pumped

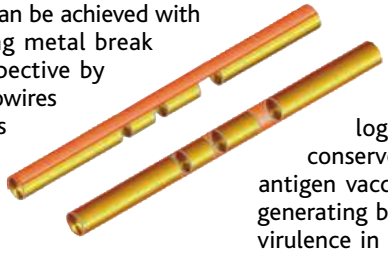
Stimulated emission, in which a photon interacts with an excited molecule and causes a second identical photon to be emitted, forms the basis for coherent light generation and amplification in lasers. The same effect was discovered in interstellar molecular clouds in the 1960s in the form of unusually bright and narrow microwave spectral lines. **Weisberg *et al.*** (p. 106; see the Perspective by **Elitzur**) report their observation of stimulated microwave emission in an OH cloud caused by photons from a distant pulsar. These results not only yield insights into maser action in interstellar clouds, but also into molecular-cloud density and distribution.

Pressure-Treated Curium

Pressure-induced delocalization of *f* electrons in rare earths and actinides involves an intimate relation between electronic configuration, structural degrees of freedom, anomalous lattice dynamics, and magnetism. A high-pressure x-ray diffraction study of curium by **Heathman *et al.*** (p. 110) revealed a sequence of structural phase transitions as its *f* electrons delocalize with increasing pressure. They identify an unusual lattice structure previously unobserved in other actinides, and on the basis of band-structure calculations, they argue that this phase is stabilized by antiferromagnetic ordering. Thus, curium joins cobalt and iron as metals that have lattice structures stabilized by magnetism.

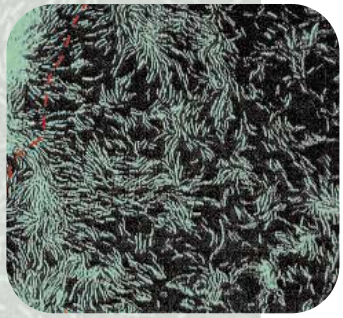
Metals with Many Gaps

The fabrication of nanostructures is facilitated not only by making small regular structures, but also by forming void spaces that can capture nanomaterials or molecules. For example, in molecular electronics, the formation of metallic gaps can be achieved with scanning probes at surfaces or by drawing metal break junctions. **Qin *et al.*** (p. 113; see the Perspective by **Martin and Baker**) created bimetallic nanowires with repeating gap structures as small as 5 nanometers by first growing bimetallic wires in porous membrane templates with thin layers of etchable metals (such as nickel within gold). After removing the templates, the wires were captured on a substrate and coated on one side with silica. After release, etching proceeded on only one side, allowing the remaining wire to stabilize the resulting gaps.



Get a Move On

Soil-dwelling myxobacteria move by a process termed gliding motility, which requires the surface expression of cellular protrusions, the type IV pili. More than 25 years ago, *Myxococcus xanthus* motility mutants lacking pili were shown to be phenotypically complemented by direct contact with motile neighbors. **Nudleman *et al.*** (p. 125) now identify the mechanism of the contact-mediated, nongenetic complementation of this type of motility. Complementation appears to be effected by the transfer from one cell membrane to another of the TGL protein, which is required for the construction of secretin pores, which in turn allow for the synthesis and retraction of the pili required for motility.



Sea-Driven Weather

Better prediction of devastating climate events, like the 2003 European heat wave, is a high priority of long-range weather forecasters. **Sutton and Hodson** (p. 115; see the news story by **Kerr**) have explored how weather depends on slowly varying environmental properties, such as basin-wide sea surface temperatures. Focusing on North America and Europe, they used a global climate model that incorporated historical records of Atlantic Ocean sea surface temperature and land-based data for pressure, precipitation, and air temperature. Ocean temperature distributions, possibly related to thermohaline circulation, have had an important influence on summertime climates on both continents and may have also influenced rainfall and drought frequency there.

The Value of Excess

The surface air temperature record of Greenland has been reconstructed mostly from analyses of the isotopic composition of H and O of the water in ice cores. A number of other factors besides average temperature can influence those proxies, however, such as the seasonality and origin of precipitation. **Masson-Delmotte *et al.*** (p. 118) measured the deuterium excess of ice from Greenland Ice Core Project (GRIP) samples in order to constrain the source and seasonality of the precipitation for the last full glacial cycle. Earth's orbital obliquity is an important control on the latitudinal temperature gradient between the source and site of precipitation, and moisture sources shifted to the south during cold periods.

Genomics and Vaccine Development

The prominent bacterial pathogen group B *Streptococcus* (GBS) is responsible for the majority of sepsis and meningitis cases between birth and 2 months of age. Based on evidence that effective maternally derived antibody protection can be transferred to newborns, different conjugate vaccines against the prevalent western serotypes are currently being assessed in clinical trials, but a rationally designed, multiunit vaccine that could broadly protect against global serotypes would be highly desirable. To identify potential antigens suitable for use in a universal GBS vaccine, **Maione *et al.*** (p. 148) scanned the genome sequences of eight GBS strains that represent the most important disease-causing serotypes. On the basis of immunological tests, GBS proteins were identified that were conserved between all strains globally. From these, a four-antigen vaccine combination emerged as the most effective at generating broad serotype immunity. Pili are often important in virulence in Gram-negative bacteria through their role in adhesion, but are usually not usually associated with Gram-positive strains such as *Streptococcus*. **Lauer *et al.*** (p. 105) nonetheless

CONTINUED ON PAGE 17

CREDITS (TOP TO BOTTOM): NUDLEMAN *ET AL.*; QIN *ET AL.*

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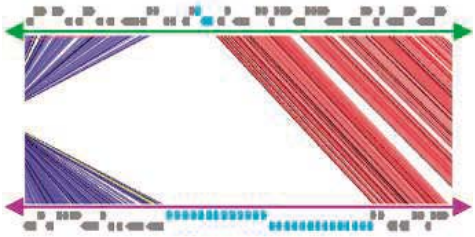
have identified pilus-like structures in GBS through immunogold electron microscopy which are composed of antigens that confer protective immunity in mouse models of maternal immunization.

The Nuclear Ins and Outs of tRNA

Transfer RNA (tRNA) is part of the machinery that converts the nucleic acid genetic code into protein. In the nucleus, tRNAs are transcribed, trimmed, and modified, and after being checked by an intranuclear quality-control system, are exported to the cytosol, ready to promote protein translation. **Takano *et al.*** (p. 140, published online 19 May 2005) now find that mature, cytosolic tRNAs are actively transported back into the nucleus by a mechanism that is independent of the usual nuclear protein import machinery that relies upon the small guanosine triphosphatase Ran. It is not clear why tRNA needs to return to the nucleus—perhaps to be subjected to further quality control, or perhaps even to promote hypothetical nuclear translation.

Theileria Genomes Work with Less

Apicomplexans are a diverse group of parasitic protozoa that cause diseases in humans and animals. *Theileria parva* is a tick-borne apicomplexan responsible for the death of 1 million cattle a year in Africa (see the Perspective by **Roos**). **Gardner *et al.*** (p. 134) present the sequence of *T. parva*, and **Pain *et al.*** (p. 131) present a comparison with the newly generated sequence of *T. annulata*. In several ways, these organisms represent stripped-down versions of more complex apicomplexans in that they have 20% fewer genes than malaria parasites; they resemble yeasts more than higher eukaryotes in the complexity of their cell cycle regulation. *Theileria* species induce transformation of lymphocytes but lack homologs of cellular protooncogenes. Other candidates that may explain the mechanism for transformation may provide drug or vaccine candidates.



Phosphorylation Rheostat

The modulation of the activity of proteins by phosphorylation has often been described as a binary switch, but **Pufall *et al.*** (p. 142) show that finer rheostat-like control can also be achieved. The transcription factor Ets-1 exhibits a graded DNA binding affinity that depends on the number of sites that are phosphorylated. Ets-1 exists in conformational equilibrium between a dynamic conformation that binds DNA and a well-folded inhibited state. Increasing phosphorylation progressively shifts the equilibrium toward the inhibited state and thus fine-tunes the level of activity. The phosphorylated region, which serves as the allosteric effector, is predominantly unstructured and flexible, and probably acts through transient interactions.

Habitat Corridors Promote Conservation

As wildlife habitats become more fragmented by human land use, wild plants and animals encounter increasing difficulties in dispersal between patches of suitable habitat. If the patches are small, then local extinctions may ensue. To mitigate this problem, conservationists favor networks of corridors to provide links between patches, but how effective is this approach? In a replicated, landscape-scale study of the role of habitat corridors in the southern United States, **Levey *et al.*** (p. 146; see the news story by **Stokstad**) followed Eastern Bluebirds as they carried native wax myrtle seeds from bushes in a central source patch to one of four surrounding receiver patches in a matrix of mature pine forest. The birds carried substantially more seeds to the corridor-connected patches than they did to the others. The authors were able to build a predictive seed-dispersal model at the landscape scale from individual-based observations on the movements of birds.

CREDIT: PAIN *ET AL.*

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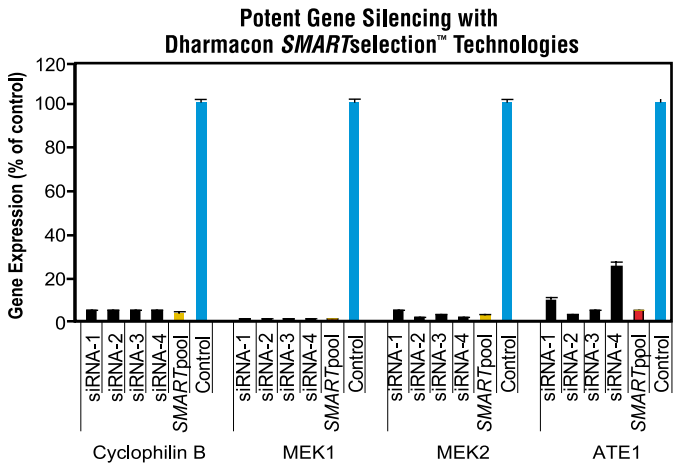

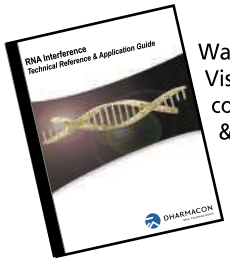

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This issue marks the 125th anniversary of *Science*, and anniversaries frequently bring our attention back to the last major one. The centennial issue emerged on 4 July 1980,* and I missed it because I was struggling with a professional transition of my own. So in preparation for this celebration, I naturally got hold of a copy as soon as I could. It's an interesting document in a number of ways. In part it looks backward—at the journal and its role in the history of science, and through splendid status reports on each of the broad research disciplines that *Science* covers. But it also looks ahead. Fred Mosteller, the distinguished statistician who was president of the American Association for the Advancement of Science (AAAS) in that centennial year, entitled his contribution "The Next 100 Years of Science." He used that essay to voice some concerns about science policy and even to make a few predictions.

Fred, never one to duck a problem, would want us to see how some of these turned out. Emphasizing the need for scientists to communicate their craft to the public, he said that AAAS's new magazine, *Science 80*, would "bring information about science to the general public." That effort, despite bold aims and an attractive format, was disappointingly short-lived. In 1980, despite a tightened academic job market, Fred could praise the U.S. National Institutes of Health for being good to young investigators. At the time, "new" investigators held 50% of competing new grants, and 23% of all awards were going to scientists under 35. Now, alas, that percentage has shrunk to less than 4%, with a huge corresponding increase in the proportion going to older researchers. In this same essay, Mosteller made some good calls. He expected more work to come from the Third World, as indeed it has. And he expressed a prescient worry about the relationship between science and government: "What began as an exuberant synthesis has become grimmer as the government presses for more paperwork and tighter accounting."

Although tempted to review the 25 years of progress since 1980, my colleagues and I went with Fred instead and decided to contemplate the future, this time by posing 25 "Big Questions" along with 100 smaller ones. The choice reflects our belief that questions are more important than answers in shaping the future of science. My love of science has much to do with its mystery; a colleague explained his own feelings by saying, "I decided I actually loved science even more than research." Research is about answers, but science is about questions, such as what is consciousness, and how could we tell, for instance, if a raven has it? Or, why are there so many more species in the tropics than in the temperate zones? (We used to say "because they're older," but it turns out that that doesn't work.)

The mental games we play in exploring questions and trying to formulate them in precise, answerable form are what gives science its special kind of intellectual fun. The essential feature of a good question is that it is ultimately testable or answerable. The Big Question that can never be wrestled with isn't worth much (that's the trouble with "intelligent design"—it's a safe harbor in terms of the testability requirement). One of the things we try to give students is the discipline that will tether their Big Question to the Big Test: Can it be answered? We hope thereby to help them avoid the fate of the postdoc whose mentor responded to his Big Idea by saying, "It isn't even wrong." In his brilliant introduction to our Big Questions, Tom Siegfried speaks of "thoroughly conscious ignorance": the state of mind that is prepared to find that important, interesting mystery whose existence had eluded us.

And the questions keep getting harder. Max Planck pointed out that each unit of new knowledge costs more than the last, because the easier answers come first and give us new techniques to apply to the next. So we are committed to asking more expensive questions that are also more difficult. Just as the progress of research creates expanding capital resource demands, it will require increased brainpower from the human resources who will pose the next questions and, eventually, answer them.

Donald Kennedy
Editor-in-Chief

10.1126/science.1115951

*This volume can by found by consulting JSTOR, an archive available to any member of AAAS through the http://aaasmember.sciencemag.org gateway.

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EDITORS' CHOICE

edited by Gilbert Chin

Kettle Lake.

GEOLOGY

Plains on Fire

Before elaborate programs of fire suppression were instituted in North America in the 20th century, wildfires occurred frequently and were critical contributors to the health and maintenance of many different ecosystems. Abundant evidence for a link between fires and climate exists for many forested regions, but less attention has been paid to nonboreal environments. The grasslands of the Northern Great Plains, which have replaced the extensive spruce forests that stood there at the start of the Holocene, are one such system.

In order to establish how drought and fire might be related in this region, Brown *et al.* constructed a 4500-year-long record of charcoal, grass pollen, and soil carbonate at Kettle Lake in North Dakota. They find that charcoal production was highest during moist intervals, when grass cover (fuel) was plentiful, and that fires did not happen at regular intervals. Spectral analysis of the data showed that for much of the late Holocene, fires recurred in cycles with a period of around 160 years, but secular trends, including any evidence of the effects of anthropogenic warming, are more difficult to detect. — HJS

Proc. Natl. Acad. Sci. U.S.A. **102**, 8865 (2005).

IMMUNOLOGY

Nippy Inoculation

For a vaccine to generate protective immunity at a level comparable to that produced by an infection, one or more secondary booster shots over an extended period of time may be required. Thus, finding ways by which the initially primed memory T cells might be more efficiently bolstered could help to increase vaccine efficacy.

Badovinac *et al.* show that mice vaccinated with dendritic cells that have been coated with peptides derived from the bacterium *Listeria monocytogenes* could mobilize a memory $CD8^+$ T cell response to a booster challenge considerably faster than mice given an attenuated bacteria vaccine. Furthermore, the dendritic cell–vaccinated mice also showed significantly greater resistance to infectious bacteria, consistent with an increased level of protective immunity. Vigorous memory responses were also generated to a range of other booster immunizations, including those from a noninfectious source, and were apparent even toward weak antigens. Vaccination with coated dendritic cells in this setting was at its most efficacious when inflammatory signals were minimal, which appeared to accelerate the rate at which $CD8^+$ T cells acquired a memory phenotype during the priming phase. — SJS

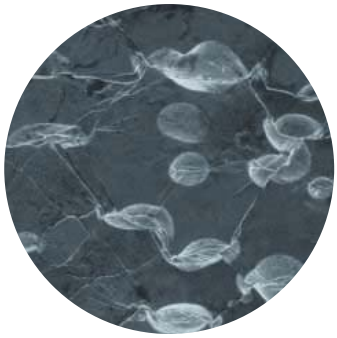
Nat. Med. 10.1038/nm1257 (2005).

MATERIALS SCIENCE

Pores for Strength

It is now possible to make bulk metallic glasses (BMGs) from a wide range of alloy compositions and to fabricate parts where the minimum dimension is at least a few millimeters. In comparison to the corresponding crystalline alloys, BMGs are almost twice as strong. Their downfall is a lack of plastic strain, which leads to softening and abrupt failure associated with shear bands, even under compression. Although they can be rendered more ductile by including a dispersed crystalline phase, this reduces the yield strength.

Wada *et al.* used four distinct hydrogenation treatments to create BMG rods with porosities between 0 and 4%. Compressive tests showed only a small decrease in the Young's modulus (about 10%), but with a strain at rupture as high as 18% and a significant increase in the rupture energy, which is the total energy under the stress-strain curve before failure. Structural analysis of the fractured samples showed that the pores acted as stress concentrators for the shear bands, causing an increase in the shear banding as the material deformed, thus increasing its toughness. — MSL



Scanning electron micrograph showing pores and shear bands.

Appl. Phys. Lett. **86**, 251907 (2005).

CHEMISTRY

Safer Sodium

Metallic sodium and potassium, as well as their alloys, are useful for their potency in chemical reduction reactions. However, their instability when exposed to oxygen is inconvenient, and their highly exothermic reaction with water is a severe fire hazard in the laboratory.

Dye *et al.* have addressed this problem by mixing the metals with silica gel. Liquid Na-K alloy combines with silica at room temperature, producing a black powder that remains air-sensitive but is easily handled under nitrogen. Heating the powder to 150°C (or heating a pure Na/silica mixture to 165°C) yields a new product, stable for months, that retains much of its reducing capacity even on exposure to dry oxygen. This so-called stage I powder can be packed in columns and used for reductions or dehalogenative couplings of eluted aromatics. Further heating of the Na–stage I powder to 400°C gives a slightly less powerful stage II reducing agent that can be handled in humid air and used for drying organic solvents or for controlled reaction with water to generate small quantities of hydrogen. Preliminary experiments suggest that stage II formation involves chemical decomposition of the silica to produce Na_4Si_4 particles. — JSY

J. Am. Chem. Soc. 10.1021/ja051786+ (2005).

PHYSIOLOGY

A Scuba Gel

In the phylogenetically ancient eumetazoan jellyfish, between the endoderm and ectoderm lies the mesoglea, a noncellular gelatinous mix of

CONTINUED ON PAGE 23

CREDITS: (TOP) BRYAN SHUMAN, DEPT GEOGRAPHY, UNIV MINNESOTA; (BOTTOM) WADA *ET AL., APPL. PHYS. LETT.* **86**, 251907 (2005)

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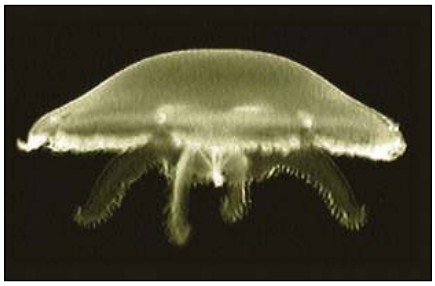

Aurelia labiata.

macromolecules that provides hydrostatic support. Thuesen *et al.* have asked whether this material might contribute in some fashion to the ability of jellyfish to thrive in eutrophic environments. Using a fiber optic oxygen probe, they detected an oxygen gradient decreasing from the convex to the concave side of the mesoglea, consistent with oxygen consumption by the metabolically active subumbrellar musculature. Furthermore, the gel appeared to be able to store substantial quantities of oxygen, enough to allow the jellyfish to survive hypoxic conditions (30% air-saturated water) and to move about vertically and vigorously in a stratified tank—100% air-saturated at the surface and only 5% saturated at 60 cm depth. — GJC

J. Exp. Biol. **208**, 2475 (2005).

MOLECULAR BIOLOGY

Enumerating Choices

Alternative splicing increases the complexity of the proteome of multicellular organisms by allowing for the generation of a large number of mRNA and protein isoforms from a relatively small number of genes. To identify alternative splicing events in the fruit fly *Drosophila melanogaster*, Blanchette *et al.* developed a microarray assay in which they examined the target pre-mRNAs of four splicing regulators. The largest number (319) of splicing events was affected by the regulator dASF/SF2, whereas the smallest number (43) was affected by PSI, suggesting that the former is a general splicing factor and the latter a more specialized one. Intermediate numbers of splicing events were affected by B52/SRp55 (107 events) and by hrp48 (90 events), and this fits well with the estimated range of 10,000 to 40,000 alternative splice junctions and roughly 200 splicing factors in *Drosophila*. In addition, cooperation was observed such that hrp48 partnered with PSI in alternative splicing events, and antagonistic regulation was also present, albeit rarely, between SR proteins (such as dASF/SF2) on the one hand and hrp48 and PSI on the other. — BAP

Genes Dev. **19**, 1306 (2005).

HIGHLIGHTED IN *SCIENCE*'S SIGNAL TRANSDUCTION KNOWLEDGE ENVIRONMENT

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Suppressor Screens

Two groups have used RNA interference (RNAi)–based screening of human cell lines to identify tumor suppressor genes. Westbrook *et al.* infected immortalized mammary epithelial cells with a retroviral RNAi library in which each RNA was tagged with a DNA barcode and looked for clones that showed anchorage-independent growth (indicative of malignant transformation). Array-based comparative genomic hybridization indicated that two genes associated with the formation of anchorage-independent colonies, *TGFBR2* (which encodes the known tumor suppressor transforming growth factor–β receptor II) and *REST* (RE1-silencing transcription factor), were frequently deleted in colorectal cancers. Knockdown of REST promoted signaling through the PI3K (phosphoinositide 3-kinase) pathway, and expression of a dominant negative form of the PI3K regulatory subunit inhibited transformation, consistent with REST acting by suppressing PI3K signaling.

Using a similar approach on immortalized fibroblasts (which can be transformed by activated RAS), Kolfschoten *et al.* identified the homeodomain pituitary transcription factor PITX1. Knockdown of PITX1 enhanced RAS signaling and produced a phenotype similar to that seen with overexpression of activated RAS. PITX1 expression was reduced in colon cancers that expressed wild-type RAS. The promoter of the GTPase-activating protein RASAL1 contained a PITX1 binding site; transfection with PITX1 enhanced *RASAL1* mRNA abundance, whereas PITX1 knockdown reduced *RASAL1* mRNA. Thus, PITX1 appears to function as a tumor suppressor that acts through RASAL1 to repress RAS signaling. — EMA

Cell **121**, 837; 849 (2005).

CREDITS: THUESEN *ET AL.*, *J. EXP. BIOL.* **208**, 2475 (2005)

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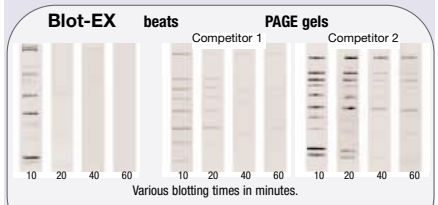

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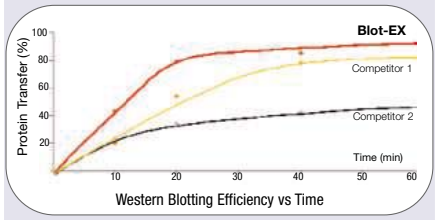

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NETWATCH

edited by Mitch Leslie

EXHIBITS

Paprika and Muscles

Luckily for science, the young Albert Szent-Györgyi (1893–1986) had a steady hand and knew anatomy. After 2 years as a frontline medic for Austria-Hungary in World War I, the future biochemist and Nobel laureate shot himself in the upper arm and blamed enemy fire. The well-placed wound liberated Szent-Györgyi from the trenches and allowed him to complete medical school.

That's one of the historical tidbits you'll find at this new biographical site. Szent-Györgyi went on to isolate vitamin C, eventually producing large quantities for research from the paprika peppers of his native Hungary. Nabbing the vitamin and discovering several steps of the Krebs cycle, the biochemical process that generates most of the cell's energy source, ATP, earned him the 1937 Nobel Prize in physiology or medicine. His career spanned continents—he worked and studied across Europe before joining the Woods Hole Marine Biological Laboratory in Massachusetts—and fields. For instance, he also identified actin, one of the proteins that power muscle contraction. Part of the National Library of Medicine's Profiles in Science series, the site stashes 72 years' worth of Szent-Györgyi's research papers, along with photos and reminiscences from colleagues.

profiles.nlm.nih.gov/WG

TOOLS

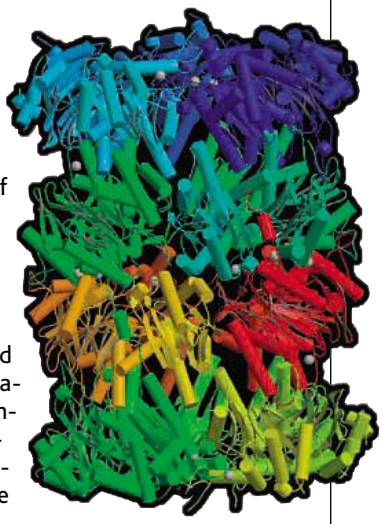
Time and Temperature

This graphing tool, created by biogeochemist Jeffrey Hicke of Colorado State University in Fort Collins, allows ecologists and other researchers to plot more than a century of U.S. temperatures without having to wrestle with often-complex climate data sets. Visitors can enter coordinates for a particular location in the lower 48 states, and the site graphs temperature anomalies—each year's deviation from the long-term average—for the nearest weather station. Plots can display maximum, minimum, and average temperatures from up to four data archives. Users can also chart aggregate values for 35 ecoprovinces, zones with similar climate and vegetation such as the chaparral of southern California.

www.nrel.colostate.edu/~jhicke/climate_data

WEB TEXT

Making Sense of Metabolism



This online text can help students keep track of the multitude of chemical reactions seething within cells. Biochemistry of Metabolism, hosted by Joyce Diwan of Rensselaer Polytechnic Institute in Troy, New York, is designed for college courses and includes plentiful diagrams, illustrations, and animations. The text's content runs from carbohydrate structure to the cleanup of worn-out proteins by the proteasome (right), the cell's garbage incinerator.

www.rpi.edu/dept/bcbp/molbiochem/MBWeb/mb1/MB1index.html

IMAGES

Plant Pinups

Despite its name, this Peruvian lily (*Scilla peruviana*; below) hails from the Mediterranean. The flower was the 2 June attraction at Botany Photo of the Day, a new site from the University of British Columbia Botanical Garden in Vancouver. Modeled after a similar NASA astronomy site, Botany Photo of the Day showcases photogenic plants from around the world, including many growing in the garden's collection.

www.ubcbotanicalgarden.org/potd

RESOURCES

Woodpecker Watch

Even kindergartners will probably be keeping an eye out for the ivory-billed woodpecker (*Campephilus principalis*) after observers this spring reported that the bird, thought to be extinct, hangs on in the swamps of eastern Arkansas (*Science*, 3 June, p. 1460). If you're setting out to look for one or think you've caught a glimpse, consult this site hosted by the Cornell Lab of Ornithology in Ithaca, New York, which is collecting reports of sightings. You'll find tips on how to distinguish the bird from the similar pileated woodpecker (*Dryocopus pileatus*), which is usually smaller and sports dark, not white, trailing edges on the wings. The site also offers extensive background on the ivory-bill's decline, including footage from a 1935 expedition to northern Louisiana that made the first recordings of the woodpecker's calls.

www.birds.cornell.edu/ivory/index.html

Send site suggestions to netwatch@aaas.org. Archive: www.sciencemag.org/netwatch

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NEWS

This Week

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FUSION RESEARCH

ITER Finds a Home—With a Whopping Mortgage

After a year and a half of tense diplomacy and secret discussions, an international fusion research collaboration has finally chosen a site for the world's most expensive science experiment. Meeting in Moscow this week, ministers from China, the European Union (E.U.), Japan, Russia, South Korea, and the United States announced that Cadarache, in southern France, has been chosen as the location of the International Thermonuclear Experimental Reactor (ITER).

"I'm extremely pleased," says Jean Jacquinot, former head of the Cadarache, fusion lab and now science adviser to France's high commissioner for atomic energy, "not because it is Cadarache, but because the whole community can now get together and build something."

Japan, after standing firm against foreign opposition, in the end may have surrendered to internal pressure to give up its desire to be ITER's host. Observers speculate that the Ministry of Finance, seeking to rein in Japan's deficit spending, may have balked at the price tag, about $2.5 billion for the host country. In return for the withdrawal of the Japanese site, companies in Japan will get substantial E.U. procurement contracts, and European money will help build a major research center in Japan. The choice of Cadarache "is disappointing," says plasma physicist Kenro Miyamoto, a professor emeritus at the University of Tokyo, "but it's preferable to having the project fall apart."

Joining forces. The E.U.'s Janez Potočnik (*left*) helps Japan's Nariaki Nakayama sign on the dotted line in Moscow.

ITER aims to recreate the sun's power on Earth. Using intense magnetic fields to hold hydrogen isotopes at enormous temperature and pressure, it would produce a flood of energy as the isotopes fuse to form larger nuclei. Originally proposed at a U.S.-Soviet summit in 1985, the ITER design was essentially complete in 2001, but when the six partners gathered in Washington, D.C., in December 2003 to pick between two candidate sites, South Korea and the United States supported Rokkasho in northern Japan, whereas Russia and China backed the E.U.'s candidate at Cadarache (*Science*, 2 January 2004, p. 22).

Further technical studies failed to resolve the impasse. Some Europeans accused U.S. officials of favoring Japan because, unlike France, it had supported the U.S.-led invasion of Iraq. The logjam began to move in April this year when E.U. research commissioner Janez Potočnik visited Tokyo; negotiations continued during a visit by Japanese Prime Minister Junichiro Koizumi to Luxembourg in May. The two rivals for host agreed on a deal guaranteeing certain concessions to the loser (*Science*, 13 May, p. 934). All that remained was for one side to back down. This week, Japan graciously removed Rokkasho from the running.

As expected, the E.U. will pay for 50% of ITER's $5 billion construction price tag. The other five partners will contribute 10% each ▶

SPACE SCIENCE

Solar-Sail Enthusiasts Say Mission Lost, Possibly in Space

Cosmos 1, a privately funded spacecraft that aimed to demonstrate solar sailing for the first time, appears never to have had a chance to unfurl its sails. But staff from the Pasadena, California–based Planetary Society, the nonprofit organization running the project, say tantalizing messages ground controllers received shortly after the craft's launch on 21 June hint that it might have made it into orbit. "We're hanging in there," says project director Louis Friedman. "But it's an increasingly dim hope."

Officials from the Russian Space Agency (RKA), which launched the spacecraft on board a converted ICBM from a submarine in the Barents Sea, believe the rocket's first stage failed, causing launcher and payload to crash into the sea. The plan was for the Volna rocket to lift Cosmos 1 into an 825-kilometer-high orbit. There researchers would have inflated booms to spread eight solar sails made of ultralight reflective Mylar, designed to show that the pressure of sunlight could slowly push Cosmos 1 into a higher orbit. The main space agencies hope to use solar sails to reach parts of the solar system inaccessible to chemical rockets (*Science*, 17 June, p. 1737). An earlier demonstration by the Planetary Society, also called Cosmos 1, failed on launch in 2001.

Although RKA's launch telemetry suggested a booster failure, some tracking stations along the planned orbit picked up signals that seemed to come from Cosmos 1. Researchers from Russia's Space Research Institute in Moscow continue to listen for the craft and are sending commands to turn on its transmitter. Even if Cosmos 1 did reach space, Friedman says, "it would be in a very low orbit and probably decayed quickly." Still, Friedman says, "it would be nice to know the spacecraft worked."

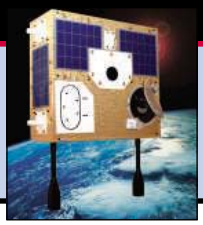
Friedman says the Planetary Society is talking to the mission's main sponsor, the entertainment company Cosmos Studios, and others about mounting another attempt. "We can still advance this whole thing," he says. But after two failed attempts, "we'll never use a Volna again."

–DANIEL CLERY

CREDIT: IVAN SEKRETAREV/AP PHOTO

38 Helium goes with the flow

41 The pulse of the Gulf Stream



43 The humble space telescope

as payments in kind. As a consolation to Japan, the E.U. will place some of its industrial contracts with Japanese companies so that Japan will end up building 20% of the reactor. Japanese researchers will make up 20% of the staff of the ITER organization, and the E.U. agreed to support a Japanese candidate for director general. Some headquarters functions will also be sited in Japan, and the E.U. promised to back Japan as a host for any subsequent commercial prototype reactor.

Japan will also get to host an extra research center to speed work toward commercial fusion reactors. Japan can choose from a list, drawn up by the six partners, that features a high-energy neutron source for materials testing, a fusion technology center, a computer simulation lab, and an upgrade of Japan's existing JT-60 fusion reactor. To pay for the center, the E.U. and Japan will contribute up to $800 million more than the normal ITER budget. "Japan will serve as what you could call a quasi–host country for the ITER project," Japan's science minister, Nariaki Nakayama, told a press conference today. "Through the [extra facility], we will become a base for international research and development in fusion energy equal in importance to the E.U."

Other partners, particularly South Korea and China, are less enamored with the deal. Luo Delong, an official with China's Ministry of Science and Technology, says that "more discussion is needed on the issues of the ITER director and the additional research facility."

European fusion researchers are delighted with the result. "Everyone is very happy," says Alex Bradshaw, scientific director of the Max Planck Institute for Plasma Physics in Garching/Greifswald, Germany, and chair of Germany's fusion research program. But some researchers are wondering whether, considering the final deal, it wouldn't have been better to be the loser—especially because France seems to be getting the whole pie, with slim pickings for other E.U. countries. There are also worries that little will be left for fusion research supporting ITER if the European research budget shrinks (*Science*, 24 June, p. 1848). "It is essential to keep other activities going, or no one from Europe will be around to use ITER" in 10 years' time, says Bradshaw.

For now, however, there's a palpable sense of relief after 18 months of wrangling. "I will certainly be quite happy to share a glass with my European colleagues," says France's Jacquinot.

–Daniel Clery and Dennis Normile

With reporting by Gong Yidong of *China Features* in Beijing and Andrey Allakhverdov in Moscow.

U.S. BUDGET

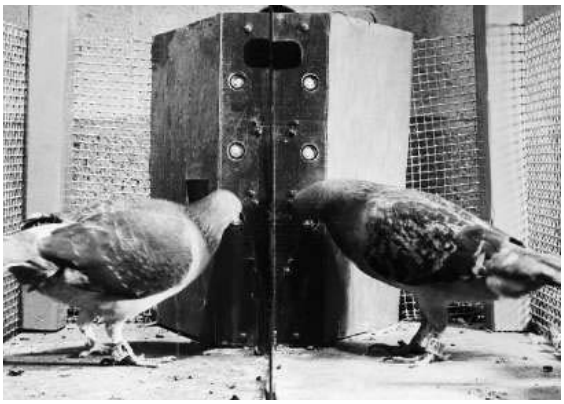
House 'Peer Review' Kills Two NIH Grants

For the second year in a row, the House of Representatives has voted to cancel two federally funded psychology grants. A last-minute amendment to a spending bill would bar the National Institutes of Health (NIH) from giving any money in 2006 to the projects, one a study of marriage and the other research on visual perception in pigeons. The grants total $644,000 a year and are scheduled to run until 2008 and 2009.

The amendment was offered by Representative Randy Neugebauer (R–TX), who last year won a similar victory involving two other grants, although his efforts were later rejected in a conference with the Senate. Researchers are hoping the Senate will come to the rescue again this year.

Neugebauer says that he is correcting skewed priorities at the National Institute of Mental Health (NIMH), in particular, the institute's "fail[ure] to give a high priority to research on serious mental illnesses." But NIH officials and scientific societies say he's meddling in a grantsmaking process that is the envy of the world. In a statement before the vote, NIH Director Elias Zerhouni called the amendment "unjustified scientific censorship which undermines the historical strength of American science."

Some House Republicans have been scrutinizing NIH's portfolio for the last few years and in 2003 almost killed several grants studying sexual behavior. Neugebauer's concerns echo the arguments of longtime NIMH critic E. Fuller Torrey, a psychiatrist who contends that the agency should spend more on diseases such as depression and schizophrenia. Last year's vote was aimed at two NIMH psychology grants that had already ended, so the effect would have been symbolic (*Science*, 17 September 2004, p. 1688).



For the birds? House lawmakers nixed a grant on perception research involving pigeons, long used in studies such as this B. F. Skinner experiment on operant conditioning.

CREDIT: BETTMANN/CORBIS

This year, the vote could have a real impact, and it came as a rude shock to the two principal investigators involved. "I'm disappointed that peer review is being undermined," says Sandra Murray of the University at Buffalo in New York, who received $345,161 from NIMH in 2005 and is expecting an equivalent amount each year through early 2009. Murray has so far enrolled 120 newlywed couples—the target is 225—in a study of factors that contribute to stable marriage and to divorce, which, she notes, "has a huge societal cost." Her study will also look at mental illnesses, she says. Neugebauer says funds for "research on happiness" would be better spent on new treatments for depression.

The second grant, to Edward Wasserman of the University of Iowa in Iowa City, continues his 14-year investigation of visual perception and cognition in pigeons. The study, slated to receive $298,688 a year through mid-2008, sheds light on "how the human brain works" and could help develop therapies for mental and developmental disorders, Wasserman says. Neugebauer, however, questions whether it "would have any value for understanding mental illnesses."

The American Psychological Association and the Association of American Medical Colleges were part of a coalition that tried last week to quash the amendment, sending a ▶

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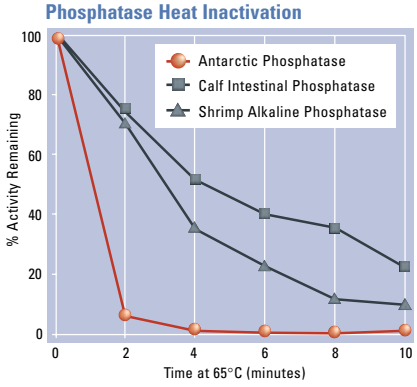

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flurry of letters to lawmakers. Several Democrats also opposed the cancellation, with Iowa Representative James Leach warning his colleagues belatedly that setting "a precedent of political 'seers' overriding scientific peers … is a slippery slope." The Neugebauer language passed as part of a set of amendments that were not debated on the floor, and no vote count was recorded.

Observers expect this year's effort by Neugebauer to be deleted (as was the case last year) when the House and Senate meet to reconcile differences in the two bills. Still, says NIMH Director Thomas Insel, "this is really unfortunate. It adds a congressional veto to the process of peer review." Adds lobbyist Patrick White of the Association of American Universities, "Our community has got to wake up on this. … We have a serious problem, and it's not going away."

–JOCELYN KAISER

2006 FUNDING

Senate Squeezes NSF's Budget

It's crunch time for the National Science Foundation (NSF). Last week, a Senate spending panel voted less money for the agency than even the president's stingy request. It delivered bleak news to backers of a proposed high-energy physics experiment at Brookhaven National Laboratory in Upton, New York. And, in a last-minute reversal, the panel restricted the agency's ability to strike the best deal on the icebreaking services needed to ferry scientists into the polar regions.

These developments are part of NSF's budget for the 2006 fiscal year that begins on 1 October. In February, the White House had requested a 2.5% budget boost, to $5.6 billion, and on 16 June the House of Representatives approved an increase of 3.1%. But the Senate panel voted a mere 1% bump. The two bills must be reconciled later this summer. "We live in hope that we'll end up better than we are now. But we know it's a tough year," says NSF Director Arden Bement.

The Senate panel did single out a few programs for special attention, including adding $6 million to the $94 million plant genome program and a similar amount for the Experimental Program to Stimulate Competitive Research to bolster 25 research-poor states. It also pumped up the $47 million operating budget of the National Radio Astronomy Observatories by $4 million.

The Senate took a harder line than did the House on NSF's $841 million education directorate, which the president had proposed cutting by $104 million. The House added back $70 million, while the Senate panel restored only $10 million. Of that, $4 million would go to a 4-year-old program linking universities and local school districts to improve student achievement that the president and the House want to shift to the Department of Education. It's seen as a marker for the Senate to lobby for retention of NSF's program.

The Senate panel took a whack at the Rare Symmetry Violating Processes (RSVP) project, a high-energy physics experiment at Brookhaven National Laboratory that would look for effects beyond the Standard Model. Citing cost estimates far beyond an initial $158 million projection, the panel withheld not only the $42 million requested in 2006 for construction but also another $14 million given to RSVP planners but not yet spent. The appropriators also told NSF that any revised version of the project would have to go back to square one in a lengthy approval process.

Tough sailing. How to find and pay for icebreaking services is one of many problems facing NSF in 2006.

Finally, the senators sided with the U.S. Coast Guard in ongoing negotiations over who should crunch the pack ice blocking entry to NSF's logistics headquarters in Antarctica, saying NSF "shall procure icebreaking services from the Coast Guard." That goes against a House preference for NSF to have "the most cost-effective means of obtaining icebreaking services." It also rewrote an earlier version of its accompanying report that ordered the Coast Guard to pay for necessary repairs to its two polar-class icebreakers, replacing it with language calling for a "joint" resolution of the issue.

–JEFFREY MERVIS AND CHARLES SEIFE

CREDIT: MICHAEL VAN WOERT/NOAA NESDIS, ORA

ScienceScope

PNAS Publishes Botulinum Paper

The *Proceedings of the National Academy of Sciences* (*PNAS*) this week published, unchanged, a hot-button paper modeling a possible bioweapons attack. And federal officials aren't happy.

The study, led by Stanford mathematician Lawrence Wein, models a terrorist attack on the U.S. milk supply using botulinum toxin and discusses possible preventive measures. *PNAS* released the paper 25 May to reporters under embargo but delayed publishing it after Department of Health and Human Services (HHS) official Stewart Simonson suggested that the information could aid terrorists and asked NAS President Bruce Alberts to hold off (*Science*, 3 June, p. 1395).

The paper is being published with only copy editing changes, writes Alberts in an editorial accompanying an online version of the paper. Data useful to a terrorist—such as the lethal dose of botulinum toxin to humans—are available on the Internet, he says, and the modeling "can be valuable for biodefense."

"While I respect the academy's decision, I do not agree with it," HHS's Simonson told *Science*. "If the academy is wrong, the consequences will be serious, and it will be HHS—not the academy—that will have to deal with them."

–JOCELYN KAISER

Barton Wants Answers

Representative Joe Barton (R–TX) has jumped into the scientific debate over the climate record of the past millennium. Citing reports in *Geophysical Research Letters* and *The Wall Street Journal* of scientific error and possible ethical lapses, the chair of the House Energy and Commerce committee is demanding that three scientists respond to detailed questions on their life's research. In 23 June letters to Michael Mann of the University of Virginia, Charlottesville, and his two co-authors on a 1998 paper, Barton requests a host of details about the climate study. These include whether the researchers performed a particular statistical test on a 15th century climate record. Mann has noted that his conclusions have been independently replicated.

Accustomed to battling his scientific critics (*Science*, 11 February, p. 828), Mann is not commenting on Barton's demands on the advice of his lawyer. Officials with the National Science Foundation and the Intergovernmental Panel on Climate Change also received letters from Barton, who wants answers by 11 July.

–RICHARD A. KERR

CLIMATE CHANGE

Senate Resolution Backs Mandatory Emission Limits

Seven years after rejecting the Kyoto climate treaty by a vote of 95–0, the U.S. Senate has affirmed the science of global warming and for the first time called for "mandatory market-based limits" on greenhouse gas emissions. The bipartisan resolution is not binding. But it repudiates the long-standing White House position that research and voluntary action are preferable to limits, and the resolution will be part of a massive energy bill approved this week by the Senate.

"The sense of the Senate is changing," says an aide to Senator Lamar Alexander (R–TN), one of 12 Republicans to support the resolution introduced by Senator Jeff Bingaman (D–NM). The statement was cosponsored by Senator Pete Domenici (R–NM), chair of the Energy and Natural Resources Committee, which plans hearings this month on a regulatory system for greenhouse gases.

The statement declares that "there is growing scientific consensus that human activity is a substantial cause of greenhouse gas accumulation." The consequences, it says, include rising sea levels, temperatures increasing at a rate "outside the range of natural variability," and more frequent and severe floods and droughts.

Before introducing his resolution, Bingaman had withdrawn a plan that would have made carbon-emitting credits much cheaper by using the 2012 emission levels as a target by 2020 and allowing the government to sell credits at a fixed price. Domenici had shown interest in the plan, but he later decided that there wasn't enough time to work out the rules. However, once Domenici stepped forward, "industry became very interested," says Paul Bledsoe, a spokesperson with the National Commission on Energy Policy, a group of scientists, policymakers, and business leaders whose recommendations last year formed the basis for the Bingaman proposal.

Chipping away. A federal energy bill now headed to conference would encourage low-emissions technologies like biomass reactors through financial incentives and tax breaks.

Passage of the nonbinding resolution followed the defeat of an emissions cap-and-trade system proposed by senators John McCain (R–AZ) and Joe Lieberman (D–CT). The plan, backed by many environmental groups, would use 2000 greenhouse gas emissions levels as a target for 2010 and set up a scheme of emissions credits; the credits then would be traded among emitters with no cost limits. This effort failed, by a vote of 60–38, for the second time in 2 years. During the debate, McCain criticized Domenici's reservations about picking industrial "winners and losers." Said McCain: "I will tell you another loser, and that is the truth." But Domenici deflected the attack: "To recognize there is a problem does not mean that [McCain's] way of solving it is the only solution."

Senator James Inhofe (R–OK) helped lead opposition to the Bingaman resolution, saying that several of its scientific assertions were "not true." Bingaman aides said that Vice President Dick Cheney called for specific textual changes, including changing the word "mandatory" to "additional." Cheney's office declined comment, although the White House has said that it opposes compulsory schemes. Inhofe's motion to block the resolution lost by a vote of 54–43.

Other aspects of the more than $36 billion energy bill passed by the Senate could cut carbon emissions if enacted. A successful amendment penned by Senator Chuck Hagel (R–NE) would authorize loans and financial incentives for companies to research carbon-cutting technologies, although those measures must be approved separately by a spending panel before any money would be available. An amendment by Senator Frank Lautenberg (D–NJ) to combat the "alteration of federal climate-change reports" was ruled out of order. It was a response to recent news that one-time White House staffer Philip Cooney, a former petroleum industry lobbyist with no science training, had edited climate science documents.

–ELI KINTISCH

FOUNDATIONS

Joining Forces for Brain Tumor Research

Frustrated by the sluggish pace of brain tumor research and the often dismal prognosis for those afflicted, eight brain tumor nonprofits* in the United States and Canada are pooling up to $6 million total to finance risky, innovative research projects, potentially including mathematical modeling and studies of neural development and stem cells. The effort announced this week, called the Brain Tumor Funding Collaborative, is unusual in the disease advocacy world, where organizations in the same disease area are typically rivals competing aggressively for donations.

Here, however, several foundations tentatively began discussing 2 years ago how to fuel brain tumor research. Roughly 41,000 people are diagnosed with brain tumors in the United States each year, and just under half of those tumors are malignant.

"We really want to break out of the traditional mold," says Susan Weiner, whose child died of a brain tumor. A cognitive psychologist and vice president for grants at the Children's Brain Tumor Foundation, Weiner notes that each of the eight groups had "to understand that you can't do it by yourself." Each has pledged a certain amount (they decline to say how much) which will enable the collaborative to offer much larger individual grants—up to $600,000 per year—than each typically funds. They will begin accepting initial proposals in August and hope to announce the first awards in January.

Brain cancer research is notoriously difficult, in part because the blood-brain barrier prevents easy access and because there's no good rodent model, says Susan Fitzpatrick, a neuroscientist and vice president of the McDonnell Foundation, another participant. But advances in genomics have begun to clarify brain cancer biology, leaving the collaborative hopeful that its effort, exceedingly challenging to pull together, says Fitzpatrick, will pay off.

–JENNIFER COUZIN

* The American Brain Tumor Association, the Brain Tumor Foundation of Canada, the Brain Tumor Society, the Children's Brain Tumor Foundation, the Goldhirsh Foundation, the James S. McDonnell Foundation, the National Brain Tumor Foundation, and the Sontag Foundation.

CREDIT: ENERGY & ENVIRONMENTAL RESEARCH CENTER

PUBLIC HEALTH

Gates Foundation Picks Winners in Grand Challenges in Global Health

In January 2003, Microsoft billionaire Bill Gates challenged scientists to think big. He asked them to identify critical problems that stand in the way of improving the health of people in developing countries, and he announced that the Bill and Melinda Gates Foundation would bankroll novel research projects aimed at solving them. Last week, after reviewing 1517 letters of intent and then inviting 445 investigators from 75 countries to submit full proposals, the foundation announced the winners: 43 projects that will receive a total of $437 million. "We all recognize that science and technology alone will not solve the health problems of the poor in the developing world," says Richard Klausner, who runs the foundation's global health program. "What science and technology can and must do, however, is create the possibility of new vaccines, new approaches, and new cures for diseases and health conditions that for too long have been ignored."

The 5-year grants range from $579,000 to $20 million and address 14 "Grand Challenges in Global Health" that mainly focus on R&D for drugs and vaccines, controlling mosquitoes, genetically engineering improved crops, and developing new tools to gauge the health of individuals and entire populations. Grant recipients come from 33 countries—although more than half live in the United States—and include Nobel laureates and other prominent academics as well as investigators from biotechnology companies and government research institutions.* "These projects truly are on the cutting edge of science, and many of them are taking very important risks that others have shied away from," says Elias Zerhouni, director of the U.S. National Institutes of Health in Bethesda, Maryland, who serves on the Grand Challenges board that evaluated the ideas.

Klausner, who formerly ran the National Cancer Institute (NCI), said the idea for the Grand Challenges grew out of a meeting he had with Gates in the fall of 2002. Says Klausner: "He asked me an interesting question: 'When you were running NCI, did you have a war room with the 10 most critical questions, and were you monitoring the progress?' " They also discussed German mathematician David Hilbert, who in 1900 famously spelled out 23 problems that he predicted "the leading mathematical spirits of coming generations" would strive to solve.

Gates announced the Grand Challenges initiative at the World Economic Forum in Davos, Switzerland, in January 2003, committing $200 million from his foundation. More than 1000 scientists suggested ideas that led the initiative's board to select 14 grand challenges (*Science*, 17 October 2003, p. 398). After sifting through the letters of intent and, subsequently, the full proposals, Gates decided to up the ante: The foundation contributed another $250 million; $27 million more came in from Britain's Wellcome Trust and $4.5 from the Canadian Institutes of Health Research.

Challengers. Richard Klausner (*left*) and Bill Gates confer at the 2003 World Economic Forum, where the initiative was launched.

Researchers applying for grants had to spell out specific milestones, and they will not receive full funding unless they meet them. "We had lots of pushback from the scientific community, saying you can't have milestones," says Klausner. "We kept saying try it, try it, try it." Applicants also had to develop a "global access plan" that explained how poor countries could afford whatever they developed.

Nobel laureate David Baltimore, who ▶

* The funded projects are listed and described at www.grandchallengesgh.org.

CREDIT: PHOTO BY REMY STEINEGGER/GCTV

ScienceScope

Researchers Consider Codes of Conduct

Scientists should adopt codes of conduct aimed at preventing the development of biological weapons. That was the consensus declared this month in Geneva at the end of a 12-day meeting of experts from 85 countries that have signed the Biological and Toxin Weapons Convention. Such codes may help raise awareness and set norms for researchers in sensitive fields, participants said. Some existing codes of conduct leave out the issue of biological weapons, and only a few scientific organizations now have such guidelines.

Although the meeting was not set up to reach any agreement, it should "help build momentum" for wider adoption of codes, says bioterror expert Ronald Atlas of the University of Louisville in Kentucky.

–MARTIN ENSERINK

Germans Resolve Funding Stalemate

BERLIN—Top universities and science organizations in Germany are applauding a long-awaited science funding boost. The 5-year, $2.3 billion "Excellence Initiative," which is designed to propel several institutions to world-class status, was blocked for more than a year by political fights between state and federal leaders (*Science*, 22 April, p. 483). The prospect of early elections this fall and a minor rewording of the proposal apparently helped break the deadlock late last month. Proposals from universities are due in September, and funds are set to flow next year. The agreement also includes a minimum 3% yearly budget increase through 2010 for Germany's nonuniversity research organizations such as the Max Planck Society.

–GRETCHEN VOGEL

Updates

■ National Institutes of Health Director Elias Zerhouni last week extended employees' deadline for reporting stock holdings by 3 months to 3 October, with a 2 January 2006 deadline for divesting. It's the second extension since a new ethics policy was announced in February.

■ The Pasteur Institute announced this week that its controversial Director Philippe Kourilsky will leave on 31 July. The institute's board of directors had decided this spring (*Science*, 22 April, p. 493) to make the change and has begun a search for his replacement.

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won a $13.9 million award to engineer adult stem cells that produce HIV antibodies not found naturally, was one of the scientists who pushed back. "At first, I thought it was overly bureaucratic and unnecessary," said Baltimore, president of the California Institute of Technology in Pasadena. "But as a discipline, to make sure we knew what we were talking about, it turned out to be interesting. In no other grant do you so precisely lay out what you expect to happen."

Other grants went to researchers who hope to create vaccines that don't require refrigeration, modify mosquitoes so they die young, and improve bananas, rice, and cassavas. In addition to HIV/AIDS, targeted diseases include malaria, dengue, tuberculosis, pertussis, and hepatitis C. Many of the projects involve far-from-sexy science. "We had this idea we were supposed to be hit by bolts of lightning," says Klausner. "But this is about solving problems. These things aren't often gee-whiz, they're one area applied to a new area."

Klausner says this is not a one-shot deal. "We're not being coy with people," he says. "If they hit all their milestones and it looks spectacular, we would expect them to come back and ask for future funding."

–JON COHEN

ECOLOGY

Flying on the Edge: Bluebirds Make Use of Habitat Corridors

In many parts of the world, landscapes are turning into isolated fragments of habitat. Conservation biologists and land managers often try to link these patches via connecting strips of habitat that, in theory, give animals better access to food and mates. But testing whether, and how, these so-called corridors work has been difficult.

On page 146, a team led by ornithologist Douglas Levey of the University of Florida, Gainesville, and ecologist Nick Haddad of North Carolina State University in Raleigh describes the largest replicated, controlled study of corridor efficacy and reports that bluebirds prefer to travel along the edges of these habitat connectors. The study also shows that small-scale observations of behavior can be used to predict how animals move through larger landscapes. Such results have conservation biologists excited. "This provides a lot more confidence that corridors are working as hypothesized," says ecologist Reed Noss of the University of Central Florida in Orlando.

The study team created eight experimental sites in the pine forests of western South Carolina to test how corridors are used. Within each, five patches of forest were cut down to make the open habitat that eastern bluebirds (*Sialia sialis*) prefer. The central "source" patch, 100 meters by 100 meters, was connected to another "receiver" patch by a 150-meter-long corridor. Each site also had three patches isolated from the source, at least one of which had "wings"—dead-end corridors on either side—in order to test the idea that even unlinked corridors help organisms find patches of natural habitat. "It's a very clever experiment," comments Stuart Pimm of Duke University in Durham, North Carolina.

The middles of the source patches were planted with wax myrtle bushes, whose fruits are a major food resource for the bluebirds. For two field seasons, Levey's postdoc Joshua Tewksbury, who is now at the University of Washington, Seattle, and others tracked single birds in the source patch as they flew from the wax myrtle bushes to other perches within patches or the surrounding forest. For each hop, until the birds flew out of sight, they noted the direction and distance traveled—usually no more than 20 meters—and the resting time at each perch. The birds' movements weren't totally random; when they encountered an edge of a patch, for example, they most often flew parallel to it.

Well-connected. Bluebirds (*inset*) used corridors to travel between patches of habitat (white rectangles) experimentally created in a pine forest (red).

The researchers then developed a computer model in which short bird flights mimicking the observational data were stitched together to simulate a 45-minute journey—the estimated time it takes a bird to digest fruit and excrete seeds—that took a simulated bird sometimes more than 250 meters from its starting point. After tens of thousands of runs, the model predicted that birds in a source patch were 31% more likely to end up in the connected patch than in unconnected ones.

To test the model, the researchers sprayed a fluorescent solution onto wax myrtle fruit in the source patches. Each week, they checked pole-mounted flowerpots in the four surrounding patches for any bird defecations with fluorescent seeds. Although they couldn't identify what kinds of birds had deposited the seeds, bluebirds were the most common species to perch over the pots.

After analyzing 11,000 defecations, they found that seeds were 37% more likely to occur in the connected receiver patch than in the isolated ones, backing up the model prediction. Also mirroring the model, there was no significant difference in seed number between the isolated patches that had the dead-end wings and those that did not, suggesting that the birds weren't using that type of corridor to find habitat patches.

Experts caution that it's difficult to generalize these results about corridor use to other species. But the basic point that small-scale observations can reliably inform landscape design is good news for those who can't afford to run large experiments. "It is comforting to conservation planners that one of the first attempts to scale up has proven quite successful," says Paul Beier of Northern Arizona University in Flagstaff.

The observations also provided insight into how bluebirds use corridors. Instead of flying down the middle, the bluebirds tended to stay along their edges in the pine plantations. The trees there may offer higher perches than the shrubby opening or better protection from hawks. One implication, for bluebirds at least, is that the width of a corridor or the quality of its habitat may not matter as much as that it has edges. Levey suspects that this edge effect holds true for other animals. But Beier points out that the experimental habitat differs from most corridors, which are usually strips of forest running through urban or agricultural land.

–ERIK STOKSTAD

CREDITS: D. LEVEY *ET AL.*, *SCIENCE*; (INSET) OLLIE KNOTT/GEORGIA FORESTRY COMMISSION, WWW.FORESTRYIMAGES.ORG

SCIENCE IN IRAN

Hard-Liner's Triumph Puts Research Plans in Doubt

TEHRAN—Shapour Etemad was stunned by the victory of Tehran's hard-line mayor Mahmoud Ahmadinejad in last week's presidential runoff election. Like many intellectuals, Etemad, director of the National Research Institute for Science Policy in Tehran, had campaigned for a moderate government, adding his name to a public endorsement of former president Hashemi Rafsanjani. After Ahmadinejad's surprise landslide victory, Etemad was left wondering if he should resign his influential post and retreat to academia.

Many Iranians were troubled by the stark choices in this election. Ahmadinejad campaigned on a promise to breathe new life into the Islamic revolution, whereas Rafsanjani pledged to seek closer ties with the United States. Although Ahmadinejad has not aired his views on science, some researchers fear that his ascendancy could result in a curtailment of foreign collaborations, an accelerated brain drain, and a shift toward more applied projects.

Unknown quantity. A proponent of the Islamic revolution, President Ahmadinejad has not made known his views on science.

That's not what Iranian scientists want to hear, given the distance they've come since 1979 when the Islamic revolution closed universities for 4 years. "We were completely isolated," says string theorist Hessamaddin Arfaei, deputy director of research at the Institute for Studies in Theoretical Physics and Mathematics in Tehran. Stagnation deepened during the protracted Iran-Iraq war in the 1980s; afterward, U.S. economic sanctions slowed the recovery.

It's only recently that Iranian science has enjoyed a widespread renaissance. The number of foreign collaborations has risen threefold in the past 4 years, says Iran's deputy minister of science, research, and technology, Reza Mansouri. "Scientific output has skyrocketed since 1993," boasts Mohammad Javad Rasaee, dean of medical sciences at Tarbiat Modares University. Iran's share of global scientific output rose from 0.0003% in 1970 to 0.29% in 2003, with much of the growth occurring since the early 1990s, according to a study earlier this year in the journal *Scientometrics*. The analysis, led by immunologist Mostafa Moin of Children's Medical Center in Tehran, was based on ▶

NANOTECHNOLOGY

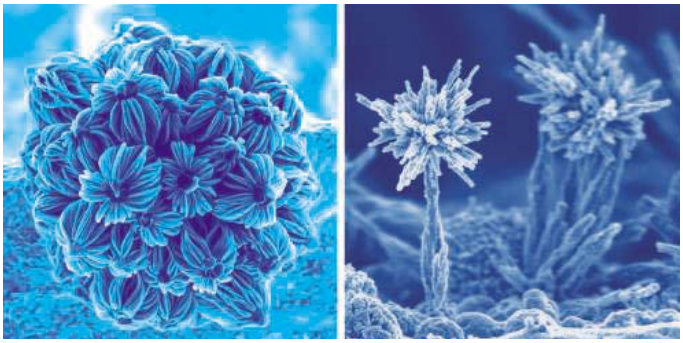
EPA Ponders Voluntary Nanotechnology Regulations

Last week, the U.S. Environmental Protection Agency (EPA) held its first public meeting to gauge sentiments about a proposed voluntary pilot program to collect information on new nanomaterials that companies are making. The agency got an earful.

More than 200 people gathered here at the Washington Plaza hotel to weigh in on the program, a possible precursor to guidelines that would mark the agency's first attempt to regulate nanotechnology. In a document released before the meeting, a coalition of 18 environmental and health-advocacy groups charged that a voluntary program would be inadequate to protect people from new chemical hazards. But most makers of nanomaterials applauded EPA's initial move as appropriate, because so little is known about the possible hazards of nanosized particles.

"The meeting was like the blind man feeling the elephant," says David Rejeski. He heads a new 2-year project at the Woodrow Wilson International Center for Scholars in Washington, D.C., on managing health and environmental impacts of nanotechnology. EPA and other agencies are still sorting out the scale of the challenge they face, Rejeski says.

Nanomaterials put regulators in an unfamiliar bind. With traditional chemical toxins, any two molecules with the same chemical formula look and behave alike. Two nanoparticles made of the same elements but of different sizes, however, may have drastically different chemical properties. Even particles of the same size and elemental composition can have very different properties, due to differences in their chemical architecture—for example, diamond nanocrystals and buckyballs shaped like soccer balls, both made of pure carbon. That diversity makes it a daunting task to sort out just which particles are hazardous to people and the environment and to control their production and release.



Hazardous? The sheer diversity of nanoparticles makes it hard to tell which ones pose risks.

As a first step, EPA is thinking about asking nanomaterials makers to submit information on just what they are producing, how much is made, and possible worker exposure. "That's a good first step," says Sean Murdoch, executive director of the NanoBusiness Alliance in Chicago, Illinois. But Jennifer Sass of the Natural Resources Defense Council in Washington, D.C., argues that asking companies to participate voluntarily doesn't go far enough. "It's going to be tough getting these companies to be good corporate citizens without the threat of regulation hanging over their heads," Sass notes.

Nearly everyone agrees that far more information is needed. To get it, some groups are starting to call for increased funding for toxicity and health studies on nanoparticles. In a commentary in the 14 June *Wall Street Journal*, Fred Krupp, president of Environmental Defense, and Chad Holliday, chair and CEO of DuPont, argued that funding for environmental health and safety studies of nanotechnology should rise from its current level of 4% to 10% of the $1.2 billion budget of the National Nanotechnology Initiative.

Rejeski argues that before a set dollar figure is agreed upon, policymakers need to decide what information they need in order to draw up nano regulations. Then, he says, they can determine how much money is needed to fill those holes. Rejeski adds that his team is currently drawing up just such an analysis and plans to release it later this summer.

–ROBERT F. SERVICE

With reporting by Amitabh Avasthi.

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publications tracked by the Institute for Scientific Information in Philadelphia, Pennsylvania. (Moin, a former science minister, ran as the sole reform candidate for president, placing fifth in the election's first round.)

But momentum is in danger of being lost, some observers warn. After Moin was eliminated from the race, Etemad and a few dozen colleagues wrote an editorial in *East* newspaper on 20 June, urging "all cultivated people" to vote for Rafsanjani and arguing that "a total catastrophe is pending and immediate."

Others caution against rushing to judgment. Mansouri anticipates only "minor fluctuations" for Iranian scientists. The situation will become clearer, he says, when the new government, including a science minister, is appointed in early August. And some found hope in last week's offer by the board of the American Institute of Aeronautics and Astronautics to suspend a controversial ban on publications from Iran and three other countries (*Science*, 17 June, p. 1722); AIAA stated it will "formally reconsider" the policy on 1 September.

Ahmadinejad's predilections may become apparent when a high council for science and technology, chaired by the president, meets this fall. The council, created earlier this year, controls most of Iran's science budget. Others argue that the country's scientific community has weathered previous changes of government well. "My thinking is that we will be affected very little, if at all," says Yousef Sobouti, director of the Institute for Advanced Studies in Basic Sciences in Zanjan. But even if some fears have been exaggerated, Etemad predicts, "we're in for a long, hard time."

–RICHARD STONE

2006 BUDGET

Can Congress Save NASA Science?

In a remarkable show of bipartisan concern, U.S. lawmakers have ordered NASA not to sacrifice research programs to pay for President George W. Bush's vision of humans on the moon and eventually Mars. But at the same time, they may have compounded NASA's problems by giving a tentative green light to Bush's plans while providing little relief for an impending budget crunch in science.

Last week, a Senate funding panel told NASA to spend an additional $400 million in its 2006 budget to fix the Hubble Space Telescope and bolster the flagging earth sciences effort. But the panel added only $134 million to NASA's $4 billion science budget to do so. Likewise, the House version of the spending bill, passed 2 weeks ago, is sympathetic to science but provides a relatively paltry $40 million increase over the president's request, most of which would go to saving the Glory earth science project. Reconciling the two pieces of legislation, one NASA manager says, "is sure to be difficult and confusing." Compounding the problem are a spate of cost overruns in research projects and growing pressure to divert money to efforts like a new human space launcher to replace the space shuttle, which is due to return to flight later this month.

NASA's new boss Michael Griffin has added another wrinkle: He's likely to rescue several science projects that the agency planned to cancel to save money. He recently ordered continued operation of the Tropical Rainfall Measurement Mission, which NASA sought to turn off last year in a decision that triggered a congressional outcry (*Science*, 13 August 2004, p. 927). NASA's efforts to win funding from the National Oceanic and Atmospheric Administration failed, so the space agency must shoulder the entire $16 million needed to keep it functioning through 2009, says NASA spokesperson Delores Beasley. Griffin is also under pressure not to turn off a host of other spacecraft, including Voyager 1 and 2, now under review for termination. Each has staunch supporters in Congress.

Griffin also recently promised senators a mission to Jupiter's moon Europa in the middle of the next decade, an effort sure to cost upward of $1 billion even with help from the European Space Agency. Congress likes the idea, and the House funding panel urged the agency to include Europa as a new start in 2007. But how that mission will fit into an increasingly strained long-term budget remains a mystery. This week, Griffin told Congress that it would be "rather dumb" to turn off Voyager 1 and 2, a cost-saving move in NASA's 2006 budget request.

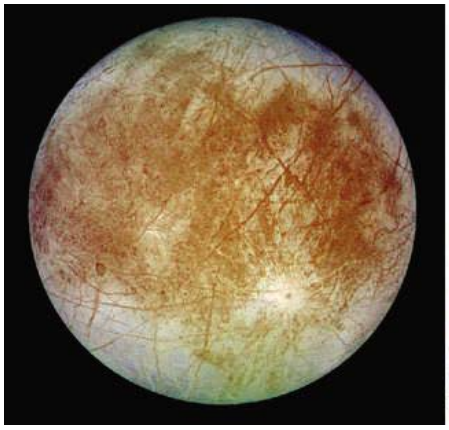
A team of agency officials and outside researchers, meanwhile, is working on ways to cope with a $1 billion cost overrun for the James Webb Space Telescope. That report is due later this summer. Cost increases in the Solar Dynamic Observatory and other missions that are already well into development are worrying agency managers.

The fate of space station science also hangs in the balance. A sweeping internal NASA study laying out a revamped construction schedule for the international space station is due in July. NASA officials say that they must decrease the 28 flights now planned to meet the president's 2010 deadline for halting shuttle flights. That change, they add, is certain to reduce the number of missions devoted to orbiting research equipment and experiments.

One likely victim, Griffin told Congress, is the centrifuge, once the central facility for station research. Life scientists will need to "go elsewhere," he says. "I cannot put microbiology and fundamental life sciences higher than" the need for a new launch vehicle for astronauts.

In contrast, preserving science aboard the station is one of the goals of a bill introduced last week to reauthorize NASA programs. "Such a restriction on the range of research disciplines aboard the [space station] is not in the best interest of the nation or of our partners," says its sponsor, Senator Kay Bailey Hutchison (R–TX). The bill calls for NASA to spend an additional $100 million on station research in the next 5 years and come up with a revamped research plan.

–ANDREW LAWLER



Shuttle diplomacy. NASA must balance competing needs, such as returning the shuttle to flight, while planning a new mission to Jupiter's moon Europa.

CREDITS (LEFT TO RIGHT): JPL/NASA; NASA/KSC

Solidified helium appears to flow without any resistance. How that happens is anything but crystal clear

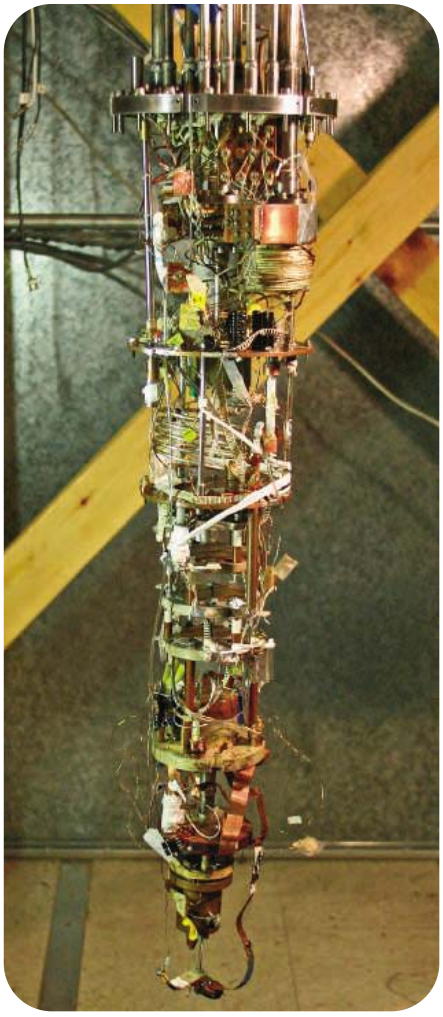
Flowing Crystals Flummox Physicists

University Park, Pennsylvania—The gizmo could be mistaken for an artifact from a science museum or a custom-made part for your old VW Beetle. An aluminum cylinder 13 millimeters wide and 5 millimeters tall sits atop a slender post of beryllium copper. From its sides, two flaps protrude like large ears on a small boy's head, and fine wires festoon the top of the can. As Eunseong Kim, a postdoc here at Pennsylvania State University (Penn State), cradles it in his palm, the device hardly looks like the heart of a breakthrough physics experiment. Yet it produced perhaps the weirdest stuff ever made.

Last year, while Kim was a graduate student, he and physicist Moses Chan used the can to squeeze ultracold helium into a crystalline solid that appears to flow without resistance—like a liquid with no viscosity. For decades physicists had mused about such a bizarre "supersolid," and others had searched for and failed to find it. So Kim and Chan's results have touched off a flurry of activity among experimenters and a debate among theorists as to whether it's even possible for a perfect crystal to flow. They are rejuvenating helium physics, a small field that has played a large role in shaping modern physics (see sidebar, p. 39).

Kim and Chan had previously seen signs of such "superfluid" flow in solid helium crammed into the pores of a spongelike glass. But on 3 January 2004 they saw the first clear evidence that it could occur in a pure crystal. "That was an exciting moment," the soft-spoken Kim recalls as he sits at his desk in the subbasement of Osmond Hall. "That morning Moses came into my lab and I said to him, 'Maybe you'll get the Nobel Prize.' "

Many others agree. "If verified, the discovery of supersolid helium will be one of the most important results in solid state physics—period," says Jason Ho, a theorist at Ohio State University in Columbus, who has come to Penn State to discuss his theory of the phenomenon. Anthony Leggett, a theorist at the University of Illinois, Urbana-Champaign, says the observations challenge the widely held belief that a well-ordered crystal cannot enter a free-flowing supersolid "phase." "I would have bet quite strongly against such a phase," says Leggett in a phone interview from his office. "It looks like the experiments will make me rethink that."



Cool! When running, Kim and Chan's cryostat hides inside a container of liquid helium.

Kim and Chan's results must still be confirmed, however. And physicists must deduce whether the helium crystal itself is flowing, or whether the effect arises from the superfluid flow of liquid helium in cracks and crevices in the crystal—a less mind-bending alternative that wouldn't count as supersolidity. To make the call, researchers are tackling new experiments that should challenge even helium physicists, who enjoy a reputation as expert tinkerers who can squeeze every drop of information out of a thimbleful of helium.

Letting go

Prone to burst into effusive laughter, Moses Chan talks fast and forcefully. But when discussing solid helium, he chooses his words carefully. "I think it's safe to say we've done all the possible control experiments," he says. "And though it sounds weird, I think the simplest explanation is that we see superfluidity in a solid." It's a big claim. Chan is saying that a material structurally similar to rock salt oozes through itself unimpeded. Yet other physicists agree with his assessment of the situation. Of course, they're used to the perversity of helium.

Since it was first liquefied nearly a century ago, physicists have puzzled over ultracold helium. Every other element freezes at some temperature, but unpressurized helium remains a liquid all the way down to absolute zero. Below 2.17 kelvin, however, the most common helium isotope, helium-4, undergoes a stranger transformation: It becomes a superfluid that flows without any resistance. That happens because, compared with other atoms, light and lively helium atoms act a bit less like billiard balls and a bit more like quantum waves. At low enough temperatures, many collapse into a single quantum wave that resists disturbances, in a process known as Bose-Einstein condensation.

Theorists have long speculated that something similar might happen in solid helium-4, which can be made by squeezing the liquid to 25 times atmospheric pressure. In 1969, Russian theorists A. F. Andreev and I. M. Lifshitz proposed that missing atoms, or vacancies, within the helium crystal could condense to form a free-flowing fluid of their own, which would mimic the flow of atoms through the liquid. But no one had seen any sign of a flowing solid until now.

To spot the stuff, Kim and Chan set their little can twisting back and forth on its shaft. The frequency of oscillation depends on the stiffness of the shaft and the inertia of the can, which in turn depends on the amount of

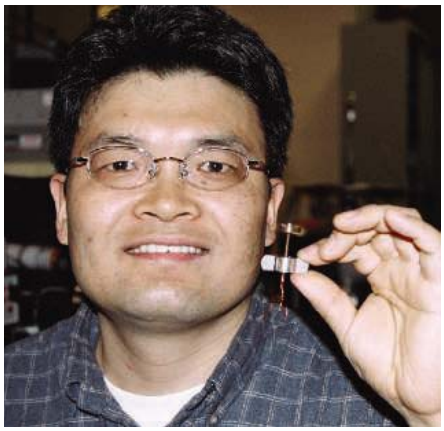
CREDITS (TOP TO BOTTOM): KENNETH EWARD/BIOGRAFX/PHOTO RESEARCHERS INC.; JOHN PASSANEAU/PENN STATE

helium stuck to it. At pressures ranging up to 145 times atmospheric pressure, the frequency began to rise suddenly as the temperature sank below about 0.2 kelvin. Those upswings indicated that as much as 1% of the helium was letting go of the oscillator and standing stock-still while the rest of the crystal twisted back and forth, as Kim and Chan reported online on 2 September 2004 in *Science*. That strange behavior implies that some of the helium glided through the crystal without resistance.

In principle, the experiment is simple. In practice, it's a challenge, as a glimpse of the guts of one of Chan's refrigerators, or "cryostats," suggests. The assemblage of tubes, wires, coils, and myriad handmade gadgets hangs like a mechanical stalactite from a platform supported by four great wooden legs. The whole thing stands inside a metal box the size of a small room, which Chan installed to block out radio interference from a nearby campus police station. From the tip of the stalactite hangs the oscillating can; when the can is twisting, its flaps move as little as a single atom's width. Kim and Chan measure changes in the oscillator's frequency to part-in-a-million precision.

Kim and Chan performed a series of experiments that slowly built the case for supersolid helium. For example, they replaced the helium-4 with the isotope helium-3, the atoms of which cannot crowd into a single quantum state because of the way they spin. That implies solid helium-3 should not flow, which is what the experimenters observed. "Moses was very careful and asked all the questions that he could ask with the kind of apparatus he had," says experimenter Richard Packard, from his office at the University of California, Berkeley. "And all the answers indicate that something lets go" in helium-4.

But although researchers agree that the experiments are sound, they disagree on how to explain them. And no one knows whether a crystal really can flow.



Twister. Eunseong Kim holds oscillator that first hinted at flow in "bulk" helium.

CREDITS (TOP TO BOTTOM): *NATURE* **141**, 243–244; A. CHO/*SCIENCE*

The Quirks and Culture of Helium

Ordinarily an inert gas so light it floats off into space, helium might seem to hold little interest for condensed-matter physicists. But since it was liquefied by Dutch physicist Heike Kamerlingh Onnes in 1908, the odd stuff has revealed much about the physics of liquids and solids. "Throughout history, it has provided a variety of new paradigms," says Jason Ho, a theorist at Ohio State University in Columbus.

Font of insight. Liquid helium has inspired key concepts in condensed-matter physics.

Since 1938, physicists have known that below 2.17 kelvin the most common isotope of helium, helium-4, becomes a "superfluid" that flows without resistance, as about 9% of the atoms crowd into a single quantum wave. In 1972, physicists discovered that helium-3 also becomes a superfluid at just a few thousandths of a kelvin. Because of the way they spin, helium-3 atoms cannot pile into a single quantum wave. Instead, they form pairs that glide without resistance, as electrons do in superconductors.

Experiments with helium-3 validated much of the "Fermi liquid theory" that also describes electrons in metals. The superfluid transition in helium-4 provided the primary test bed for the theory of "second-order phase transitions," which describes, for example, the onset of magnetism in materials.

While helium has helped theorists develop key concepts, experimenters working with ultracold helium have developed a reputation for old-fashioned ingenuity. Their experimental devices are usually mechanical contraptions that shake, spin, and squeeze helium to produce subtle but telling signals. By tradition, "you don't buy your instrumentation; you invent it," says John Goodkind, an experimenter at the University of California, San Diego. "You make it, you leak-check it, and you fix it."

Helium physicists are also known for seat-of-the-pants problem solving, slathering their refrigerators with soap and glycerin to plug elusive leaks so small only superfluid helium squeezes through, or using a condom to regulate the flow of gas.

Never very big, the field of helium physics has contracted since its heyday in the 1970s. But researchers trained in helium physics have become leaders in high-temperature superconductivity, nanomechanical devices, two-dimensional electron systems, and other areas. "The people in the field are willing to take risks," says Richard Packard, an experimenter at the University of California, Berkeley. "They aren't afraid of making new devices, and when they go out into other fields, that same state of mind goes with them." **–A.C.**

Exchange, grains, and defects

Ohio State's Ho has no doubt that it can. He has come to Penn State to discuss the theory he is developing, which assumes that supersolid flow occurs and attempts to explain how swirls resembling smoke rings reduce the flow as the temperature increases. In a conference room on the third floor of Davey Hall, Ho stands beside a viewgraph projector and gestures at the screen with a length of half-inch threaded steel rod. "If it gets too complicated, then I apologize," he says. He's been talking for 90 minutes and will go on for another hour. He's covered blackboards on two walls with equations. It seems supersolidity has no easy explanation.

Theorists have already advanced several ideas, but most run afoul of the data in one way or another. For example, Andreev and Lifshitz's notion of a quantum wave of vacancies jibed nicely with the results of Kim and Chan's experiments with helium in porous glass, reported in January 2004 in *Nature*. It seemed plausible that, cramped by the nanometer-sized pores, the crystals would be riddled with vacancies. But this scheme appears less likely in the "bulk" crystal, as experimental evidence suggests that pure solid helium has very few vacancies. And if the vacancies are mobile, then they should quickly wander to the edge of the crystal and vanish, anyway.

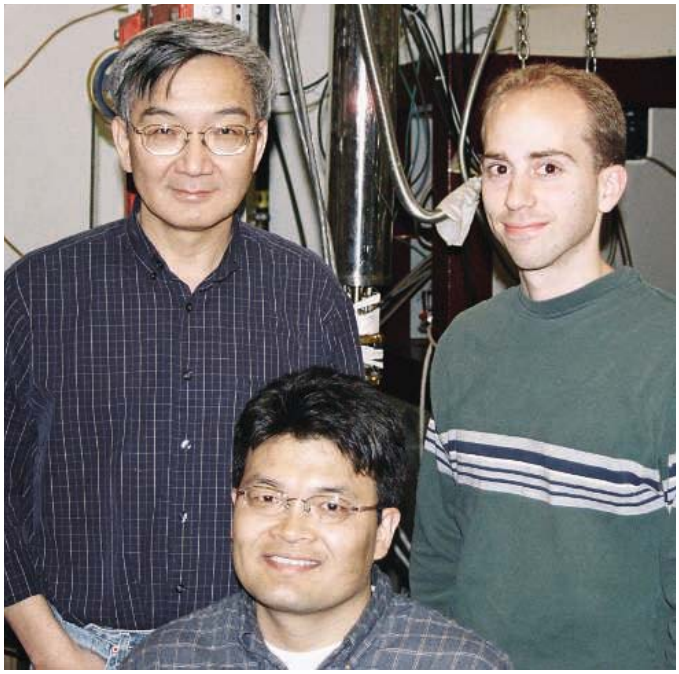
If vacancies don't explain the flow, then perhaps some of the helium atoms themselves undergo Bose-Einstein condensation within the crystal. Leggett and others explored that idea in the 1970s. At first it sounds absurd: In a crystal, each atom is ordinarily confined to a specific site in the three-dimensional "crystal lattice," whereas in the quantum wave of a Bose-Einstein condensate it's impossible to say precisely where any particular atom is. But thanks to their quantum-wave nature, neighboring helium atoms have a tendency to swap places spontaneously in a process called "exchange." If they trade places often enough, then in principle some of them may

be able to collapse into a single wave and flow in a way that leaves the pristine crystal structure unsullied.

In actuality, that scenario may be unlikely, however. Leggett originally calculated that the amount of free-flowing helium would be tiny. And computer simulations suggest that a perfectly orderly helium crystal does not undergo Bose-Einstein condensation, as theorists David Ceperley of the University of Illinois, Urbana-Champaign, and Bernard Bernu of Pierre and Marie Curie University in Paris, France, reported in October 2004 in *Physical Review Letters*. "There's been a lot of speculation that somehow you can get a flow of atoms in a solid," Ceperley says in a phone interview. "I just don't think that's possible."

Some theorists have suggested that supersolid helium is really superslushy helium, with the flow occurring in liquid seeping between tiny bits of solid. Boris Svistunov and Nikolay Prokof'ev of the University of Massachusetts (UMass), Amherst, note that solid helium undoubtedly consists not of a single large crystal but of many smaller interlocking crystalline grains. They calculate that more conventional superfluid liquid flowing between the grains might account for Kim and Chan's data, as they reported this April in *Physical Review Letters*. But that explanation would require a multitude of micrometer-sized grains, whereas data indicate that helium tends to form fewer, larger grains.

The secret to supersolidity could lie in the conceptual middle ground between a flowing crystal and liquid flowing between crystal grains, says theorist Burkhard Militzer from his office at the Carnegie Institution of Washington, D.C. The flow could occur within the crystal, he speculates, but along elongated, immobile defects called "stacking faults," which resemble missed stitches in a piece of fabric. Simulations show that atoms swap places easily along the faults, Militzer says, but they do not yet prove that such faults account for Chan's observations.



Shaking things up. Moses Chan (left) and colleagues Eunseong Kim (center) and Anthony Clark have theorists debating whether a crystal can flow.

More data, please

To sort things out, physicists are planning a variety of experiments designed to confirm the observation—and to explain why others had failed to spot the effect before.

In 1981, researchers from Cornell University in Ithaca, New York, and Bell Telephone Laboratories in Murray Hill, New Jersey, saw no evidence for supersolidity in torsional oscillator experiments similar to Chan's. But the researchers may have been foiled by bad luck and a bit of helium-3. The experiment most comparable to Chan's was contaminated with several parts per million of helium-3, says Cornell's John Reppy in a phone interview. "That would have been enough to wipe out the signal, according to Moses's [data]," he says. "I'm willing to believe it." Reppy and colleagues at Cornell are running yet another torsional oscillator experiment to try to reproduce the effect.

Others have searched for supersolidity by trying to squeeze solid helium through tiny holes. If the crystal is free-flowing then it might seep through, just as superfluid liquid helium will flow through openings so small they stop ordinary liquid helium dead. But the fact that solid helium cannot perform this trick may mean only that supersolid and superfluid helium respond to pressure differently, says John Beamish, an experimenter at the University of Alberta in Edmonton, Canada: "If it is a supersolid—and we're not saying it isn't—it doesn't flow as you would naively expect."

Curiously, over the past decade, experimenters studying the propagation of sound and heat in solid helium did see signs of a "phase transition." But they interpreted them very differently. John Goodkind and colleagues at the University of California, San Diego, found that the speed of sound in solid helium increases suddenly as the temperature drops below 0.2 kelvin, and the rate at which the waves die away peaks at that temperature. Goodkind interpreted these and other signs as evidence that some sort of defect proliferates as the temperature of the crystal increases and that these "defectons" undergo Bose-Einstein condensation *above* a critical temperature.

Goodkind hopes to resume his experiments, and Haruo Kojima, a physicist at Rutgers University in Piscataway, New Jersey, has begun sound experiments of his own. If supersolidity exists, then it should be possible to generate a sound wave in which only the free-flowing helium moves, explains Kojima, who has come to Penn State to discuss the experiments with Chan. But the experiments may be tricky, he warns, because researchers aren't sure precisely what signals they should expect to see.

For his part, Chan is devising an elaborate experiment to determine just how many vacancies, grain boundaries, and defects exist in a helium crystal. He plans to run a torsional oscillator in the beamline of a synchrotron x-ray source and to alternately shake the crystal and shine x-rays through it. The sloshing of the oscillator will tell how many atoms are in the crystal, while the scattered x-rays will reveal how many lattice sites there are in it. Only if the crystal is perfect will the two numbers be equal. The experiment may be the key to cutting through the confusion, says UMass's Svistunov in a phone interview: "To answer, how perfect is the crystal? In my opinion, that is the most important question in the field."

Meanwhile, in the sunless subbasement of Osmond Hall, Chan's young colleagues continue their work. Kim is taking data with a bigger oscillator that twists at lower frequencies. Graduate student Anthony Clark is studying solid hydrogen. In March, at the American Physical Society meeting in Los Angeles, California, Clark presented preliminary data that suggest hydrogen may also become a supersolid (*Science*, 8 April, p. 190). "I want to be completely confident," Clark says, "and we've been doing a lot of control experiments."

Both Kim and Clark say they feel intense pressure working on such potentially groundbreaking experiments. Chan takes the hubbub in stride, however. "Nobel Prize or no Nobel Prize, that doesn't matter. What's really nice is that [our work] has attracted so much attention" from other researchers, he says. "We have already had more fun than we deserve." He smiles wryly, like a magician who has pulled off a particularly clever trick. Only this time, not even the conjurer knows precisely how the trick works.

–ADRIAN CHO

CREDIT: A. CHO/*SCIENCE*

Atlantic Climate Pacemaker for Millennia Past, Decades Hence?

An unsteady ocean conveyor delivering heat to the far North Atlantic has been abetting everything from rising temperatures to surging hurricanes, but look for a turnaround soon

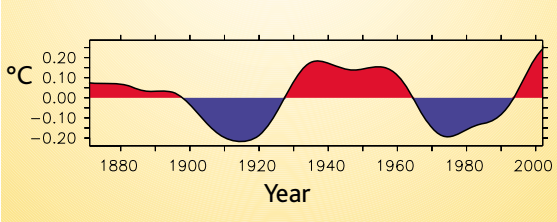
Benjamin Franklin knew about the warm Gulf Stream that flows north and east off the North American coast, ferrying more than a petawatt of heating power to the chilly far North Atlantic. But he could have had little inkling of the role that this ponderous ocean circulation has had in the climatic vicissitudes of the greater Atlantic region and even the globe.

With a longer view of climate history and long-running climate models, today's researchers are tying decades-long oscillations in the Gulf Stream and the rest of the ocean conveyor to long-recognized fluctuations in Atlantic sea-surface temperatures. These fluctuations, in turn, seem to have helped drive the recent revival of Atlantic hurricanes, the drying of the Sahel in the 1970s and '80s, and the global warming of the past few decades, among other climate trends.

The ocean conveyor "is an important source of climate variability," says meteorologist James Hurrell of the National Center for Atmospheric Research in Boulder, Colorado. "There's increasing evidence of the important role oceans have played in climate change." And there are growing signs that the conveyor may well begin to slow on its own within a decade or two, temporarily cooling the Atlantic and possibly reversing many recent climate effects. Greenhouse warming will prevail globally in both the short and long terms, but sorting out just what the coming decades of climate change will be like in your neighborhood could be a daunting challenge.

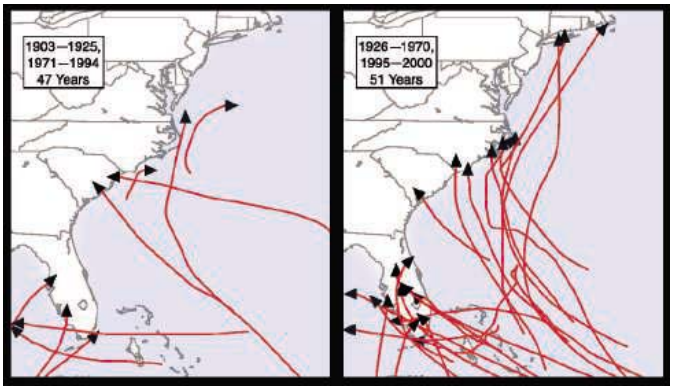
Researchers agree that the North Atlantic climate machine has been revving up and down lately (*Science*, 16 June 2000, p. 1984). From recorded temperatures and climate proxies such as tree rings, researchers could see that temperatures around the North Atlantic had risen and fallen in a roughly 60- to 80-year cycle over the past few centuries. This climate variability was dubbed the Atlantic Multidecadal Oscillation (AMO). Ocean observations suggested that a weakening of the ocean conveyor could have cooled the Atlantic region and even the entire Northern Hemisphere in the 1950s and '60s, and a subsequent strengthening could have helped warm it in the 1980s and '90s. But the records were too short to prove that the AMO is a permanent fixture of the climate system or that variations in the conveyor drive the AMO.

Taking the long view, climate modeler Jeff Knight of the Hadley Centre for Climate Prediction and Research in Exeter, U.K., and colleagues analyzed a 1400-year-long simulation on the Hadley Centre's HadCM3 model, one of the world's leading climate models. The simulations included no changes in climate drivers such as greenhouse gases that could force climate change. Any changes that appeared had to represent natural variations of the model's climate system.



Wobbly ocean. North Atlantic temperatures have wavered up and down at a roughly 60- to 80-year pace.

At April's meeting of the European Geosciences Union in Vienna, Austria, Knight and colleagues reported that the Hadley Centre model produces a rather realistic AMO with a period of 70 to 120 years. And the model AMO persists throughout the 1400-year run, they note, suggesting that the real-world AMO goes back much further than the past century of observations does. The model AMO also tends to be in step with oscillations in the strength of the model's conveyor flow, implying that real-world conveyor variability does indeed drive the AMO.



Bad warmth. The AMO's warm years favor more U.S. hurricanes (*right*).

Such strong similarities between a model and reality "suggest to me it's quite likely" that the actual Atlantic Ocean works much the same way as the model's does, says climate modeler Peter Stott of the Hadley Centre unit in Reading, who did not participate in the analysis. Hadley model simulations also support the AMO's involvement in prominent regional climate events, such as recurrent drought in North East Brazil and in the Sahel region of northern Africa, as well as variations in the formation of tropical Atlantic hurricanes, including the resurgence of such hurricanes in the 1990s.

On page 115, climate modelers Rowan Sutton and Daniel Hodson of the University of Reading, U.K., report that they could simulate the way relatively warm, dry summers in the central United States in the 1930s through the 1960s became cooler and wetter in the 1960s through 1980s. All that was needed was to insert the AMO pattern of sea-surface temperature into the Hadley atmospheric model. That implies that the AMO contributed to the multidecadal seesawing of summertime climate in the region.

If the Hadley model's AMO works as well as it seems to, Knight and his colleagues argue, it should serve as some guide to the future. For example, if North Atlantic temperatures track the conveyor's flow as well in the real world as they do in the model, then the conveyor has been accelerating during the past 35 years—not beginning to slow, as some signs had hinted (*Science*, 16 April 2004, p. 371). That acceleration could account for about 10% to 25% of the global warming seen since the mid-1970s, they calculate, meaning that rising greenhouse gases haven't been warming the world quite as fast as was thought.

Judging by the 1400-year simulation's AMO, Knight and colleagues predict that the conveyor will begin to slow within a decade or so. Subsequent slowing would offset—although only temporarily—a "fairly small fraction" of the greenhouse warming expected in the Northern Hemisphere in the next 30 years. Likewise, Sutton and Hodson predict more drought-prone summers in the central United States in the next few decades.

But don't bet on any of this just yet. The AMO "is not as regular as clockwork," says Knight; it's quasi-periodic, not strictly periodic. And no one knows what effect the

CREDITS (TOP TO BOTTOM): R. T. SUTTON AND D. L. R. HODSON, *SCIENCE*; S. B. GOLDENBERG *ET AL.*, *SCIENCE*

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strengthening greenhouse might have on the AMO, adds Sutton. Most helpful would be an understanding of the AMO's ultimate pacemaker. In the Hadley Centre model, report modelers Michael Vellinga and Peili Wu of the Hadley Centre in Exeter in the December *Journal of Climate*, the pulsations of the conveyor are timed by the slow wheeling of water around the North Atlantic. It takes about 50 years for fresher-than-normal water created in the tropics by the strengthened conveyor to reach the far north. There, the fresher waters, being less dense, are less inclined to sink and slide back south. The sinking—and therefore the conveyor—slows down, cooling the North Atlantic and reversing the cycle.

That may be how the Hadley AMO works, says oceanographer Jochem Marotzke of the Max Planck Institute for Meteorology in Hamburg, Germany, but it doesn't settle the mechanism question. How a model generates multidecadal Atlantic variability "seems to be dependent on the model you choose," he says. Before even tentative forecasts of future AMO behavior are taken seriously, other leading models will have to weigh in, too.

–RICHARD A. KERR

Astronomy

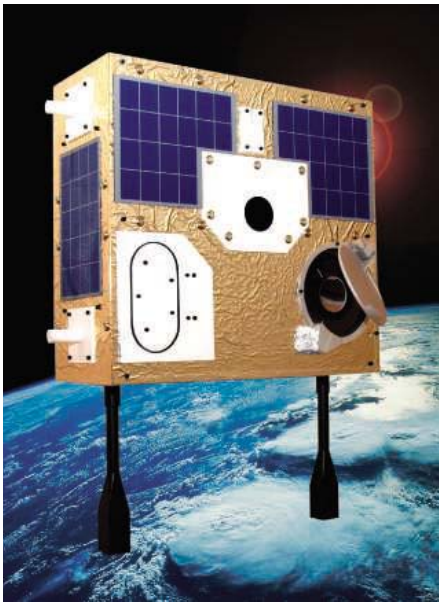
Suitcase-Sized Space Telescope Fills a Big Stellar Niche

Small but single-minded, Canada's MOST microsatellite is revealing the inner clockwork of stars and characterizing exoplanetary systems

MONTREAL, CANADA—To astronomers, bigger telescopes usually mean better telescopes. But a Canadian space-based instrument is bucking that trend. Just 2 years into monitoring subtle periodic dips in starlight, the suitcase-sized MOST (Microvariability and Oscillations of Stars) telescope is probing the hidden internal structures of sunlike stars and pinning their ages down to a greater precision than ever before. At a meeting here,* astronomers announced that MOST has also begun to provide information about the planets that orbit some of those stars, even hinting at their weather patterns. "Not bad for a space telescope with a mirror the size of a pie plate and a price tag of $10 million Canadian, eh?" says astronomer Jaymie Matthews of the University of British Columbia.

MOST blasted into space aboard a converted Russian intercontinental ballistic missile on 30 June 2003. Nicknamed "the Humble Space Telescope," Canada's first space observatory is also the world's smallest, weighing in at only 60 kg and sporting a modest 15-cm mirror. Designed and built for less than 1/20 of the projected cost of any upcoming competing mission, the single-purpose satellite does without most of the instruments found on its larger space-based cousins but still conducts science no other orbiting observatory is equipped to do.

Above the blurring effect of our atmosphere, MOST's ultraprecise photometer can detect fluctuations in stellar brightness as small as one part in a million—10 times better than ground-based telescopes can achieve. Thanks to a specially designed gyroscope, the Canadian Space Agency–run microsatellite can stare at a star around the clock for up to 2 months. The Hubble Space Telescope, by contrast, can look at a given object for only about 6 days. "MOST is pushing frontiers in stellar astronomy in terms of time sampling and light-measuring precision," Matthews says. "While this may seem more abstract than what Hubble can do, it is just as revolutionary in terms of what this tiny telescope allows us to see in stars and their planets."



Packed with potential. Boxy MOST focuses on doing one thing very well.

CREDIT: E. KOELEMEYER/MACMILLIAN SPACE CENTRE

Using methods of asteroseismology—the study of starquakes—MOST monitors optical pulsations caused by vibrations of sound waves coursing through a stars' deep interior. Just as geologists can map Earth's interior from earthquake signals, astronomers can probe a star's hidden structure by tracking minute oscillations in its luminosity. As the star contracts, its internal pressure increases, heating its gases and temporarily increasing its brightness. The MOST team hopes the technique will lead to better theories about how stars evolve with age.

"Most of the research is being done on sunlike stars, because we know how to interpret the data using our sun as a model," says starquake hunter Jørgen Christensen-Dalsgaard of the University of Aarhus in Denmark. According to astrophysical models, stars between 80% and 170% as massive as the sun pass through the same basic life cycles as the sun does and should show similar upper atmosphere turbulence and micromagnitude oscillations. But whereas short, subtle changes in brightness are relatively easy to detect on the sun, they are much trickier to spot in more-distant sunlike stars.

Not until 2000 did ground-based telescopes become sensitive enough to confirm them in a few dozen solar-type stars. Those observations used spectroscopes to detect shifts in the color of light, from which astronomers could calculate the radial velocity of the stellar surface as it moves up and down. Now MOST—which makes it possible to draw similar inferences from much smaller changes in brightness—is opening a new chapter in the field, says astronomer Travis Metcalfe of the High Altitude Observatory in Boulder, Colorado: "This modest instrument is bound to have a great impact on our understanding of stellar evolution."

In July 2004—a year into its observations—MOST's science team, led by Matthews, generated their own waves in the asteroseismology community when they published their observations on the well-studied star Procyon. To the shock of everyone, the satellite found that Procyon showed none of the oscillations that ground-based measurements had seen and theoretical models had predicted for nearly 20 years. "We had 32 continuous days of data representing over a quarter of a million individual measurements and saw nothing," says Matthews.

Asteroseismologists around the world are still puzzling over those observations. Christensen-Dalsgaard, a member of one of

* CASCA 2005, Montreal, Quebec, 15–17 May.

the first teams to detect Procyon's oscillations from the ground and biggest critic of MOST's Procyon results, suspects that either light scattered back from Earth into the telescope washed out the data, or "noisier"-than-expected convection in the star's atmosphere made the oscillations unreadable. The possibility of using MOST to study stars' atmospheric churning "is, of course, itself interesting," he adds. The MOST team revisited Procyon this year and plans to publish an analysis of the new measurements within a few months.

Things went more smoothly this year, when MOST fixed its gaze on Eta Bootis. This time the data matched both stellar models and earlier ground-based observations. By comparing the data against a library of over 300,000 theoretical stellar models, Matthews and his team have pegged the star's age at 2.4 billion years, plus or minus 30 million years—about 10 times the precision of previous estimates. Studying a variety of sunlike stars with differences in mass, age, and composition will lead to better models, Christensen-Dalsgaard says.

As a bonus, MOST's ability to measure exquisitely small variations in starlight enables it to double as an exoplanet explorer. At the meeting, the MOST team announced that the telescope had staked out an alien world around a far-off star and turned up subtle hints of an atmosphere and possible cloud cover. NASA's Spitzer Space Telescope had detected the infrared glow from exoplanet HD209458b in March. MOST tracked the subtle dip in optical brightness as the planet slipped behind its parent star during its orbit.

By following the frequencies and amplitudes of the changes in stellar brightness, the team concluded that the planet is a gas giant 1.2 times as massive as Jupiter, parked less than 1/20 as far from its star as Earth is from the sun. Astronomers think HD209458b's low reflectance (less than 40%, compared with 50% for Jupiter) sets limits on the planet's atmosphere, in which the Hubble Space Telescope saw signs of carbon and oxygen in 2004. MOST will conduct a 45-day survey of the system later this summer with the hope of getting a clearer picture of the exoplanet's atmosphere and even its weather: temperature, pressure, and cloud cover.

MOST's asteroseismological monopoly is destined to be short-lived. Similar satellites on the horizon include the European COROT (Convection, Rotation, and planetary Transits) mission, slated for launch in June 2006, and NASA's own planet seeker, Kepler, due in 2007. Unlike MOST, both satellites will be technologically capable of detecting Earth-size worlds. COROT's more sensitive detector will also be able to look at many stars simultaneously, rather than one at a time, as MOST does. But COROT and Kepler will focus on fainter stars than MOST observes, and their vision will be limited to smaller sections of the sky, Metcalfe says. As a result, he argues, during the tail end of its 5-year life span, MOST will complement the other missions and will not become obsolete when they come on line.

Christensen-Dalsgaard agrees. "MOST is giving us the experience that we need to learn how stars behave photometrically and helps us learn how to choose targets for these later missions," he says. "So in the next couple of years, we need to make the most out of MOST."

–ANDREW FAZEKAS

Andrew Fazekas is a freelance writer in Montreal, Quebec.

Central Asia

Combating Radioactive Risks And Isolation in Tajikistan

The science academy of this war-weary country is reaching out for help in tracking down lost radioactive sources—and restoring scientific vitality

FAIZABAD, TAJIKISTAN—In the early 1990s, as civil war raged in this mountainous land, a terrorist's prize was here for the taking. Powerful radioactive sources lay buried in an open-air, gravel-covered pit on a compound ringed by a dilapidated concrete wall and chain-link fence. During the 5-year war, villagers and fighters pillaged nearby apple orchards and industrial sites. But the makings of dirty bombs—including radioisotopes such as cesium, cobalt, and americium in old Soviet gauges and other devices—remained untouched. "We were lucky," says Gennady Krivopuskov, manager of the 6-hectare waste storage facility 50 kilometers northeast of the capital, Dushanbe. "Maybe the radiation hazard signs kept looters away."

How long the rad cops' luck will last is an open question. One or two derelict radioactive generators, which produce electricity from the heat harnessed from the decay of strontium-90, were never moved to this storage facility and remain unaccounted for, experts say. Each radioisotope thermoelectric generator (RTG) packs a whopping 40,000 curies—equivalent to the radioactivity from strontium-90 released during the 1986 Chornobyl explosion and fire. "How serious is it that they aren't secured? Well, that depends on who has them," says a Western diplomat. Last month, a U.S. Department of Energy (DOE) team was in Dushanbe to train specialists at the Nuclear and Radiation Safety Agency of the Academy of Sciences of the Republic of Tajikistan (AST) on how to detect abandoned sources. Search efforts are about to get under way.

Concern about RTGs as a serious proliferation threat first got attention 3 years ago,

Isolation. Barriers have been upgraded at the Faizabad radwaste site, with help from the U.S. Department of Energy.

CREDIT: R. STONE

Shock and Recovery

DUSHANBE—The hunt for hot sources (see main text) is one of several challenges that the Academy of Sciences of the Republic of Tajikistan (AST) faces as it attempts to recover from a brutal civil war that followed the Soviet collapse. Some of the academy's prized assets, including a cosmic-ray physics laboratory, astronomical observatories, and a network of seismic stations, emerged surprisingly intact. But lingering memories of the civil war and ongoing concerns about Tajikistan's anemic law enforcement—including an unsolved car bombing outside a government building last January—have put a damper on international cooperation.

During the Cold War, the Soviets bankrolled some high-profile Tajikistani projects. The Soviet Equatorial Meteor Expedition from 1968 to 1970, organized by AST's Institute of Astrophysics, painted a detailed picture of meteor bombardment of Earth and wind patterns in the upper atmosphere. And in 1963, the Institute of Earthquake Engineering and Seismology inaugurated the Lyaur testing range, a unique facility where artificial earthquakes—simulated with explosives—probed the durability of full-scale model buildings constructed from novel seismic-resistant materials and designs.

By the early 1990s, however, most scientific activity in Tajikistan, the poorest of the 15 nations born from the old Soviet Union, had ground to a halt. During the worst years of the civil war, in 1992 and 1993, food was scarce, power outages frequent, public transportation virtually nonexistent, and the water supply and telephone lines unreliable. "Yet we came to work every day," says Alla Aslitdinova, director of AST's central library. "I can't explain why." Thefts were commonplace. "People stole our computers and other equipment," says Khursand Ibadinov, director of the astrophysics institute. "Fortunately, they left the telescopes," he says, including a 40-centimeter Zeiss astrograph at the Hissar observatory near Dushanbe.

Star trackers. Khursand Ibadinov, director of the astrophysics institute (*left*), and academician Pulat Bobojonov with a high-precision astrometry telescope at the Hissar observatory.

Shelling, gunfire, and penury were not the only problems. The Russian government asserted ownership of the Murgab cosmic-ray research station, perched in the Pamir Mountains northeast of Dushanbe. Because of the dispute—which shows no signs of ending—"for 14 years no experiments have been carried out there," says AST president Mamadsho Ilolov, a mathematician.

The country's seismic stations, meanwhile, require an extensive upgrade from analog to digital instruments. But the investment would be worth it, says David Simpson, president of IRIS, a university seismological consortium based in Washington, D.C.: It could sharpen the monitoring of regional seismic hazards and help probe fundamental questions such as the geological structure of the Pamirs. Simpson led a seismological project at Nurek reservoir in Tajikistan from 1975 into the early 1980s. "Even under Soviet power at that time, it was a wonderful, friendly, and beautiful place to live and work," he says.

AST researchers hope to soon end the isolation that has cocooned them since the civil war. "I have a dream to start academic and student exchanges" with U.S. universities, says Aslitdinova, who spent 4 months as a Fulbright scholar late last year at Northwestern University in Evanston, Illinois. It's a dream many Tajikistanis share, but one that will be a struggle to make come true. **–R.S.**

when the International Atomic Energy Agency (IAEA) in Vienna helped secure a pair of abandoned generators in the Republic of Georgia (*Science*, 1 February 2002, p. 777). IAEA has since learned that more than 1000 such generators were produced in the Soviet Union; the vast majority stayed in Russia, where they were used primarily to power Arctic lighthouses. But in recent years scores have gone astray or been vandalized for scrap metal. In Tajikistan, where the generators were used to power remote weather stations, four RTGs have been recovered and are awaiting transfer to Russia for disposal, says Ulmas Mirsaidov, director of the radiation safety agency. Although Mirsaidov told *Science* that all RTGs in Tajikistan are now secured, DOE officials and a Western diplomat in Dushanbe say that units are missing; one or two is the best estimate based on present information.

Tajikistan's radiation agency is now working with IAEA to compile an inventory of radiological sources. "We're helping them make sense of their records and develop a search plan," says Carolyn MacKenzie, a radiation source specialist with IAEA. There's no indication that any RTGs have fallen into the wrong hands. Still, there's a disconcerting lack of knowledge about where precisely to look. "When the Soviets left, the records weren't passed on," MacKenzie says. "We don't have definite information," adds Roman Khan, a health physicist at Argonne National Laboratory in Illinois. DOE's Search and Secure Program, Khan says, has provided Mirsaidov's agency with a suite of instruments—including a portable radiometer capable of detecting alpha and beta particles and gamma rays, a hand-held gamma ray spectrometer, and a broad energy germanium detector—for tracking down orphan sources.

The hope is that the loose RTGs can be located and stored as soon as possible at the Faizabad facility, a hilly territory alive with discus-sized tortoises, a cacophony of sparrows, and a riot of bright-red poppies. A short walk up the road, through an inner fence patrolled by a machine gun–toting guard, is a whitewashed building with a massive gray steel door. Buried here, 9 meters beneath the dirt floor, are a variety of radioactive sources, including x-ray fluorescence instruments containing americium-241 that were used for geological surveys, radiotherapy canisters filled with cobalt-60, and four RTGs recovered so far.

The repository was rebuilt last year with DOE and U.S. State Department support. The previous structure was frail indeed: On several occasions, high winds that sweep down from the mountains from September to March tore off the corrugated steel roof, says Krivopuskov, who after 26 years of service receives a salary of $12 per month. Thanks to the renovations, he claims, the sources "can stay here safely for 1000 years." In the meantime, though, he and his colleagues must contemplate the fate of the sources that haven't yet been secured.

–RICHARD STONE

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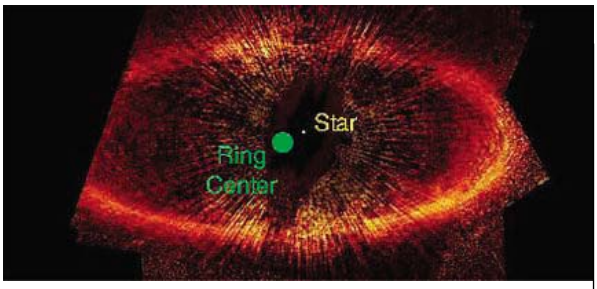
RANDOM SAMPLES

Edited by Constance Holden



A Planet's Unseen Hand

This image from the Hubble Space Telescope, released last week by NASA, shows the young nearby star Fomalhaut surrounded by a dusty ring created by comet and asteroid collisions. The star is far from the center, which suggests that gravity from an unseen planet is displacing the ring.

Creationism Skirmish

In the face of a libel suit, the head of an organization that tracks the ongoing battle over teaching Darwin in schools has agreed to publicly acknowledge errors in a recent article.

This spring, Eugenie Scott, director of the National Center for Science Education (NCSE) in Oakland, California, published an article in *California Wild*, the magazine of the California Academy of Sciences, mentioning that lawyer Larry Caldwell had proposed the names of two creationist books to his local school board and quoting a scientist accusing him of "gross misunderstanding" of science.

In April, Caldwell slapped Scott and the center with a libel suit. Although it does not mention the magazine, editor Keith Howell agreed to remove the online link to Scott's article and to publish a letter from Caldwell as well as a mea culpa from Scott. The latter acknowledges that Caldwell did not introduce the two books, and that the comment about misunderstanding science referred to someone else.

"I think there's a danger in lumping everyone in one category," says Caldwell, complaining that NCSE has in the past wrongly labeled him a "creationist activist." Caldwell says he believes in intelligent design.

Scott says she stopped calling Caldwell a creationist after he objected. She points to the suit as contributing to "an absolute explosion" of evolution-related "flare-ups" in state or local education systems. NCSE has counted 71 in 33 states so far this year, compared to a past annual average of between 50 and 60.

Who Donates Organs?

More than half the U.S. adult population has pledged to donate their organs after death—an increase of 26% since 1993, according to Gallup poll data presented last week to the Washington, D.C.–based Institute of Medicine's committee on increasing rates of organ donation.

The poll found that readiness to donate one's organs varies depending on age, ethnicity, education, and income, but that males and females are equally likely to donate. The survey of 1900 people revealed that more than two-thirds of adults between 35 and 44 years old are ready to donate, compared with 55% of those between 18 and 24, and only 38% of people over 65. Many people do not realize that the organs of older patients can still make for valuable transplants, said bio-ethicist Laura Siminoff of Case Western Reserve University in Cleveland, Ohio.

Whereas about 62% of whites and Asians are willing to donate organs, the figure falls to 47% among Hispanics and 25% among blacks. "Among African Americans, there is a high level of distrust with not just organ donation but also the medical system," said Siminoff. And this is the group waiting the most for organs. Committee member Clive O. Callender, a surgeon at Howard University in Washington, D.C., noted that African Americans make up 13% of the U.S. population but represent 35% of those on waiting lists for kidneys, the most commonly transplanted organ.

Gene Knockout Leaves Mice Squeakless

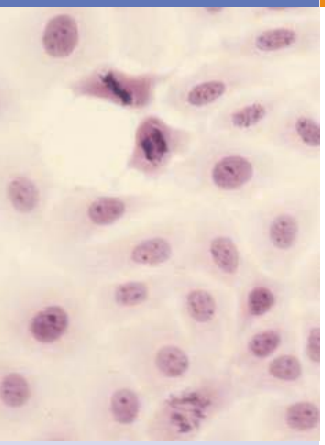
A new study suggests that the mouse version of a human "speech gene" also plays a key role in murine communication. It adds to evidence that the gene may be widely involved in animal communication.

In humans, mutations in the *FOXP2* gene cause impairments in both understanding and motor control of speech. The gene differs only slightly in mice, so researchers led by neuroscientist Joseph Buxbaum of the Mount Sinai School of Medicine in New York City bred two types of *FOXP2* "knockout" mice: One group was homozygous—that is, it had two disrupted copies of the gene; the other group had one functional and one defective gene.

The homozygotes did not make the ultrasonic sounds that young mice emit when separated from their mothers, Buxbaum's team found. Moreover, they had severe motor defects and died young. Even those with one normal copy of the gene had problems, making significantly fewer sounds than did normal mice when separated from their mothers, the scientists reported last week in the *Proceedings of the National Academy of Sciences*. Examination of brain tissue revealed abnormalities in the cerebella of the double knockouts, especially in the Purkinje cells, which are involved in fine motor control.

Simon Fisher of the Wellcome Trust Center for Human Genetics at Oxford University in the U.K., who helped isolate the gene in humans, cautions that the knockouts may not offer a "direct parallel" with human speech problems from *FOXP2* disruption. But he says such studies "will be critical for gaining insights into the neuromuscular pathways regulated by this gene."

CREDITS (TOP TO BOTTOM): NASA/ESA/P. KALAS *ET AL.*; UC BERKELEY; JOE MCDONALD/CORBIS



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PEOPLE

Edited by Yudhijit Bhattacharjee

JOBS

Hubble's new boss. Astronomer Mattias "Matt" Mountain has been named director of the Space Telescope Science Institute (STScI) in Baltimore, Maryland. The job involves taking responsibility for what may be NASA's highest-profile science missions: overseeing both the Hubble Space Telescope and its planned successor, the James Webb Space Telescope (JWST). Mountain, 49, is currently director of the Gemini Observatory, which runs twin 8-meter telescopes in Hawaii and Chile.

The move is a natural for Mountain, who is the telescope scientist for the 6.5-meter JWST and co-chairs a review of the mission's proposed science program in light of soaring budget estimates (*Science*, 13 May, p. 935). He must also deal with the merits and costs of extending Hubble's life and bridging the gap between the two missions.

"Matt's experience both with Gemini development and Gemini operations will be a great asset in helping us make that transition," says outgoing STScI Director Steven Beckwith. "It's going to be quite a challenge," admits Mountain, who assumes his post on 1 September.

CELEBRITIES

Flying high. South Korean geneticist Woo Suk Hwang earned iconic status in his country earlier this year when he became the first researcher to clone human embryonic stem cells bearing the genetic imprint of diseased patients (*Science*, 20 May, p. 1096). Now the nation's flagship airline is rewarding him with a decade of free travel for himself and his wife.

"This will give me more chances to attend scientific conferences," says the Seoul National University professor, who availed of the offer last month to attend a 2-day meeting on stem cell research at Baylor College of Medicine in Houston, Texas. The couple may fly in the highest service class available when traveling in connection with Hwang's research. "In the event that he needs to travel with research supplies, Korean Air will consider accommodating cargo transport needs," according to a 3 June press release that announced the offer.

NONPROFIT WORLD

No small change. Portugal's wealthiest man has left behind a generous endowment aimed at making the country a magnet for biomedical research.

The $585 million Champalimaud Foundation was created last month with a single donation from businessman Antonio Champalimaud, who died last year at the age of 86. His will names former Portuguese health minister Leonor Beleza as president of the foundation, which will support excellence in all fields of biomedicine. "We intend to link pure scientific research with clinical research," Beleza says.

The foundation, based outside Lisbon, will have a small staff to run a grants program. It will also award a $1 million biennial prize for vision research as a tribute to Champalimaud, who lost his eyesight late in life. Members of the preliminary advisory group include immunologist Antonio Coutinho, neuroscientist Antonio Damasio, former Irish president Mary Robinson, and former European Parliament President Simon Veil.

Canadian leave-taking. The chiefs of Canada's two main science agencies are stepping down from their posts this summer—but on quite different terms.

Thomas Brzustowski (below), president of the Natural Sciences and Engineering Research Council, is departing voluntarily for academia after consecutive 5-year terms. The 68-year-old aeronautical engineer says his departure shouldn't affect a strategic planning exercise that the agency hopes will result in a doubling of its budget: "This isn't a one-man show."

By contrast, Marc Renaud (above right), was denied a chance to serve a decade as president of the Social Sciences and Humanities Research Council and to resolve an imbroglio over the council's strategic direction. Some Liberal Party sources attribute the decision to the government's displeasure over Renaud's criticism of its research funding policies. "I was frustrated, but now I'm thinking it was a blessing because it forces me to reposition myself before I'm 60," says the 59-year-old sociologist, who had taken office in 1997. Replacements have yet to be named.

DEATHS

Climate science pioneer. Atmospheric chemist Charles Keeling, who was the first to confirm that the burning of fossil fuels was leading to an accumulation of carbon dioxide in the atmosphere, died of a heart attack at his home in Montana on 20 June. He was 77.

An inventor departs. Electrical engineer and Nobelist Jack Kilby, who took the world into the computer age by inventing the integrated circuit, died on 20 June at the age of 81.

Got any tips for this page? E-mail people@aaas.org

CREDITS (TOP TO BOTTOM): AP; K. PU'UOHAU-PUMMILL/GEMINI OBSERVATORY; MARTIN LIPMAN/LIPMAN STILL PICTURES; SOURCE: NSERC

THE EUROPEAN CLIMATE FORUM

A Platform for Open-Minded Debate on Climate Change

I. What is dangerous climate change?

In October 2004, the European Climate Forum convened more than 60 scientists – coming from countries all around the globe – in Beijing to assess scientific evidence relevant to the question: What constitutes dangerous climate change? This question is essential for climate policy because the UN Framework Convention on Climate Change, the UNFCCC, defines as the overarching goal of climate policy the prevention of dangerous interference with the climate system. The UNFCCC is valid international law, ratified by even more states than the Kyoto Protocol, including the U.S.

The question is difficult because the very concept of danger requires a combination of factual and normative statements. So far, however, science has evolved by trying to avoid, rather than cultivate, such a combination. Meeting the challenge of climate change will require scientific progress to overcome this barrier. The mission of the European Climate Forum is to do so by fostering debate involving scientists and a variety of stakeholders, in particular from business and the world of NGOs.

II. A Reasonable Benchmark

A key outcome of the Beijing symposium was the collection of new evidence supporting the view that dangerous climate change is constituted by global warming of more than 2 degrees Celsius over pre-industrial levels for a long period of time.

Recent research on ice sheets and glaciers shows that such long-term warming would involve the risk of a sea-level rise of about 5 meters. This would be due to the combination of thermal expansion of the oceans with the melting of ice in mountainous regions, in Greenland, and in Antarctica.

According to current scientific knowledge, there are several more large-scale risks involved in a sustained warming of more than about 2 degrees, including the risk of losing the Amazon rainforest because of changing precipitation patterns. To be effective, climate policy needs a clear and simple benchmark. The 2 degrees criterion provides this benchmark.

III. An Ongoing Process

The European Climate Forum is a platform for joint studies on climatic change. It brings together representatives of different parties concerned with the climate problem:

- energy industries,
- companies engaged in renewables,
- major energy users,
- insurance and finance,
- policy-makers,
- environmental NGOs,
- and scientists.

Faithful to its mission, the European Climate Forum will not try to enforce a consensus on the 2 degrees view of dangerous climate change among its members or a broader public. Rather, we will continue the inquiry on how to avoid dangerous climate change by organizing modeling exercises, case studies and debate with partners characterized by different opinions, but also by mutual respect for these opinions.

A key tool for this process will be the Kyoto-Plus Lab. Under this label, ECF has agreed with the Heinrich Böll Foundation to organize a process of inquiry on how to move from the Kyoto Protocol to a long-term climate policy consistent with the 2 degrees goal. It will involve workshops, discussion papers, publications in the scientific literature and the mass media, and a web-based communication platform involving innovative tools like board and computer games – the latest product in this series being the board game "Winds of Change" (see pictures below).

photographs: h@imagery.nu / www.imagery.nu

For more information see:

www.European-Climate-Forum.net

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LETTERS

Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 6 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

What Can Be Done To Stop the Decline?

DONALD KENNEDY'S EDITORIAL "TWILIGHT for the Enlightenment?" (8 Apr., p. 165) is an articulate summary of one of the most frightening aspects of "progress" in America. I find this epoch in U.S. history quite discouraging, quite frightening, and indicative of a decline and fall of American science and culture. But what is missing in so many summaries is the question, what is to be done?

In my work on K–12 education, I know how easy it is to argue the failure of public education to prepare graduates for life and advocacy in the 21st century. Perhaps the political challenge illustrated by the Editorial and the education challenge that I have been working on can be joined. I suggest assembling CEOs of companies such as Intel, IBM, Motorola, and Microsoft; selected university presidents; scientists; and educators, i.e., those whose profit and joy rest upon rationality, to coherently press for the required dramatic revision of U.S. education.

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Arguing About the Use of Stem Cells

IN HIS EDITORIAL "TWILIGHT FOR THE Enlightenment?" (8 Apr., p. 165), Donald Kennedy makes an interesting ethical argument in defense of (presumably embryonic) stem cell research. By pointing out that beliefs regarding the beginning of a human life are not universal, the author implies that there is no basis for restricting research in the area. If one were to use this yardstick of universal objection to determine when research becomes unethical, it follows that even the infamous medical experiments of the Nazis might pass muster. Rather than being the exclusive domain of Christian fundamentalists, concern over the ethical implications of embryonic stem cell research is widespread and is an area of academic interest (*1*). I respectfully submit that the author could more effectively support his position by discussing the point of true contention: when human life begins. Certainly the author would not deny his adversaries the skepticism that he so strongly advocates?

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Evangelical Biologists and Evolution

IN HIS EDITORIAL "TWILIGHT FOR THE Enlightenment?" (8 April, p. 165), Donald Kennedy expresses concern that the teaching of evolution is being contested in 40 states. Even though I consider myself an evangelical Christian, I, too, share that concern. I was educated in a fundamentalist school where a literal 6-day creation period was taught. Yet over the years, I've come to accept Darwinian evolution.

The evangelical Christian public may be mostly anti-evolutionary, but that may not be true of evangelical Christian biologists. Curious as to how these biologists deal with evolution and creation, I wrote to "the Professor of Biology," at the 104 schools of the Council of Christian Colleges and Universities listed in the CCCU's Web site. Biologists from six schools refused to participate. Sixty-seven schools responded. Twenty-five percent of the respondents affirmed their belief in a young Earth and a 6-day creation period. Twenty-seven percent hold the theistic evolution position, which accepts the common descent of all living things and believes that God acts through natural laws. The remainder were either reluctant to take a specific stance or were what are called old Earth progressive creationists—Earth is billions of years old, but God acted creatively to bridge the gaps, i.e., between amphibians and reptiles and between reptiles and birds. Five of this group merely sent printed statements of their school's position affirming its belief in a Creator God, but that there are multiple ways in which he might have done it.

Although deeply divided in their views of evolution and creation, there is what I think is a small but significant trend among fundamentalist Christian biologists toward accepting Darwinian evolution. Hopefully, it will continue and spread to the fundamentalist public.

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Debating Whale Sanctuaries

IN REVIEWING THE INTERNATIONAL WHALING Commission's whale sanctuaries, L. Gerber and co-authors take a purely ecological viewpoint ("Do the largest protected areas conserve whales or whalers?", Policy Forum, 28 Jan., p. 525). Other key considerations when considering the justification for sanctuaries and their boundaries are that these are practical management measures that, as noted by Ludwig *et al.* (*1*), need to "include human motivation and responses as part of the system to be studied." The decision in 1994 to declare the Southern Ocean a sanctuary for whales was taken in light of the difficulties of regulating whaling in this remote area after revelations that the former Soviet Union had caught protected species for 30 years while systematically falsifying records (*2*). The boundaries for the sanctuary also recognized geopolitical realities and locations of past catches by the Antarctic whaling industry as well as ecological considerations.

A pair of melon-headed whales breach the surface off Reunion in the Indian Ocean.

Whales have a special status in international law as highly migratory species, and decisions as to whether they should be exploited must be shared by all countries and not just those who wish to kill them (*3*). In particular, although the IWC has a unique competence to regulate whaling in all waters, it is customary for the views of range states to be taken into account in the designation of sanctuaries. The Indian Ocean sanctuary was adopted with full support from the range states, and the Southern Ocean Sanctuary, which has few range states, was adopted with overwhelming support (26 votes in favor, with only Japan voting against). Even Japan has accepted the decision for almost all species by limiting its formal objection only to minke whales.

The science underlying the design and operation of marine protected areas has advanced considerably since the IWC

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sanctuaries were adopted, and Gerber *et al.* make many valuable suggestions for enhancing the effectiveness of these sanctuaries. More recent sanctuary proposals, such as those presented by Brazil and Argentina for a South Atlantic Whale Sanctuary, have already incorporated the development of a management plan to address these issues.

VASSILI PAPASTAVROU[1] AND RUSSELL LEAPER[2]
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3. Article 64 of the UN Convention of the Law of the Sea.

Response

PAPASTAVROU AND LEAPER MAKE AN IMPORTANT point that IWC whale sanctuaries are established for a number of often nonscientific reasons. As commissioned by the IWC Scientific Committee, our review of the Southern Ocean Sanctuary (SOS) examined the costs and benefits of the establishment of the SOS from a scientific perspective only. Our position remains, however, that IWC sanctuaries—while demonstrating initial goodwill toward preservation of whale stocks—are flawed and that their continued application in their present configuration does little more than provide a false sense of security, by assuming that protections for whale populations are in place.

The science of marine reserves has improved considerably in the past decade. Globally, reserves are being established on the basis of clear ecological principles, seeking measurable objectives, and using sound management plans to achieve these objectives. There does not appear to be any desire by either pro- or anti-whaling nations to incorporate these novel concepts into the management of the IWC sanctuary program. The 2004 IWC meeting in Sorrento, Italy, exemplifies the gridlock gripping the IWC sanctuary program. Anti-whaling nations have co-opted the program into a means to exclude whaling in advance of the application of the Revised Management Procedure/Revised Management Scheme (RMP/RMS), which would replace the current moratorium on commercial whaling, and pro-whaling nations are funding the participation of developing nations in the IWC to garner votes to defeat sanctuary proposals. Meanwhile, scientific whaling continues to occur in sanctuaries. This is an untenable situation that will only be resolved by the implementation of the RMP/RMS, which should compel IWC members to review existing and planned sanctuaries. We agree that IWC sanctuaries could be effective tools for the conservation and management of marine resources; however, without significant changes to the sanctuary program by IWC members, sanctuaries will remain "paper parks" serving little ecological purpose.

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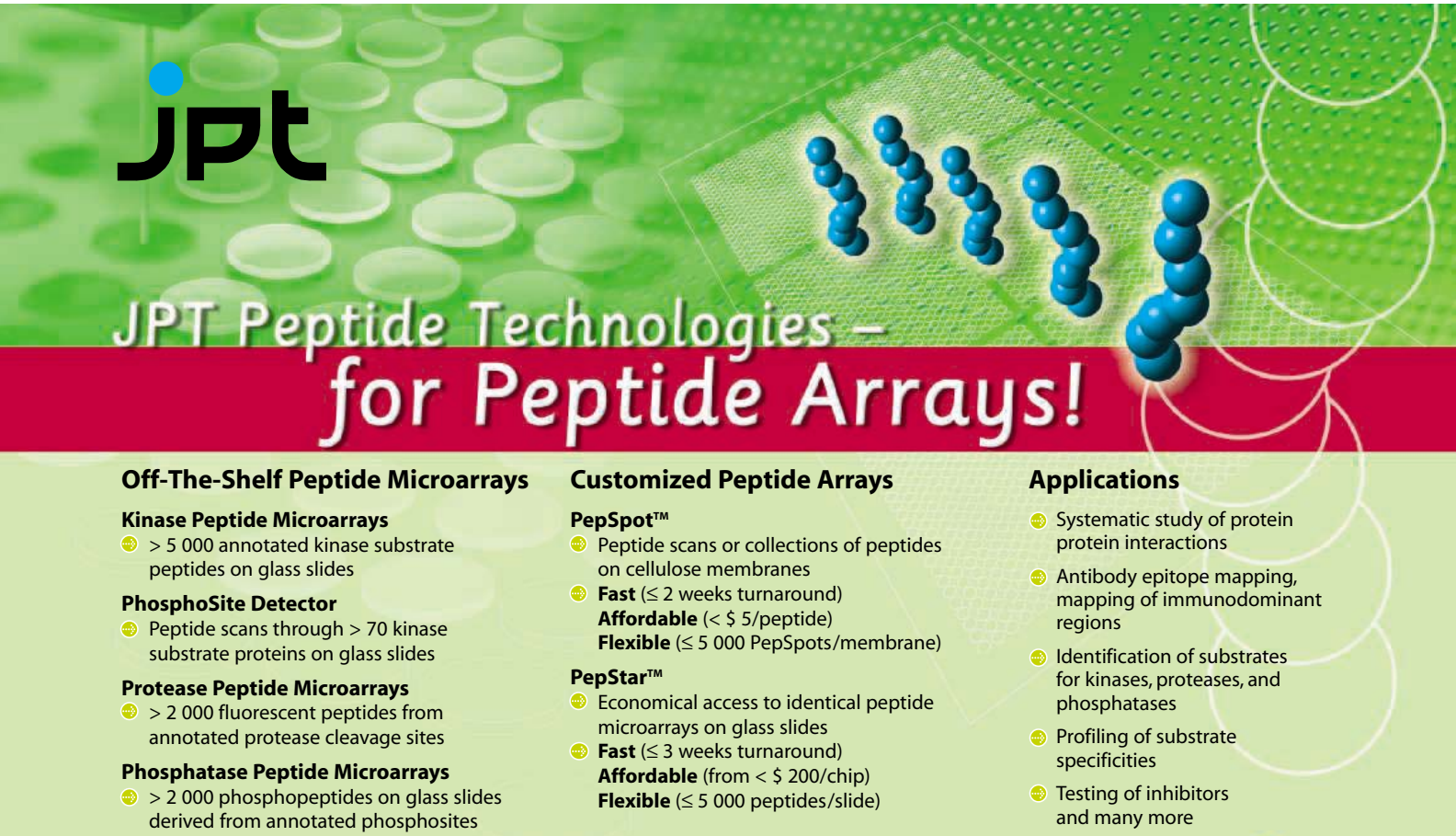
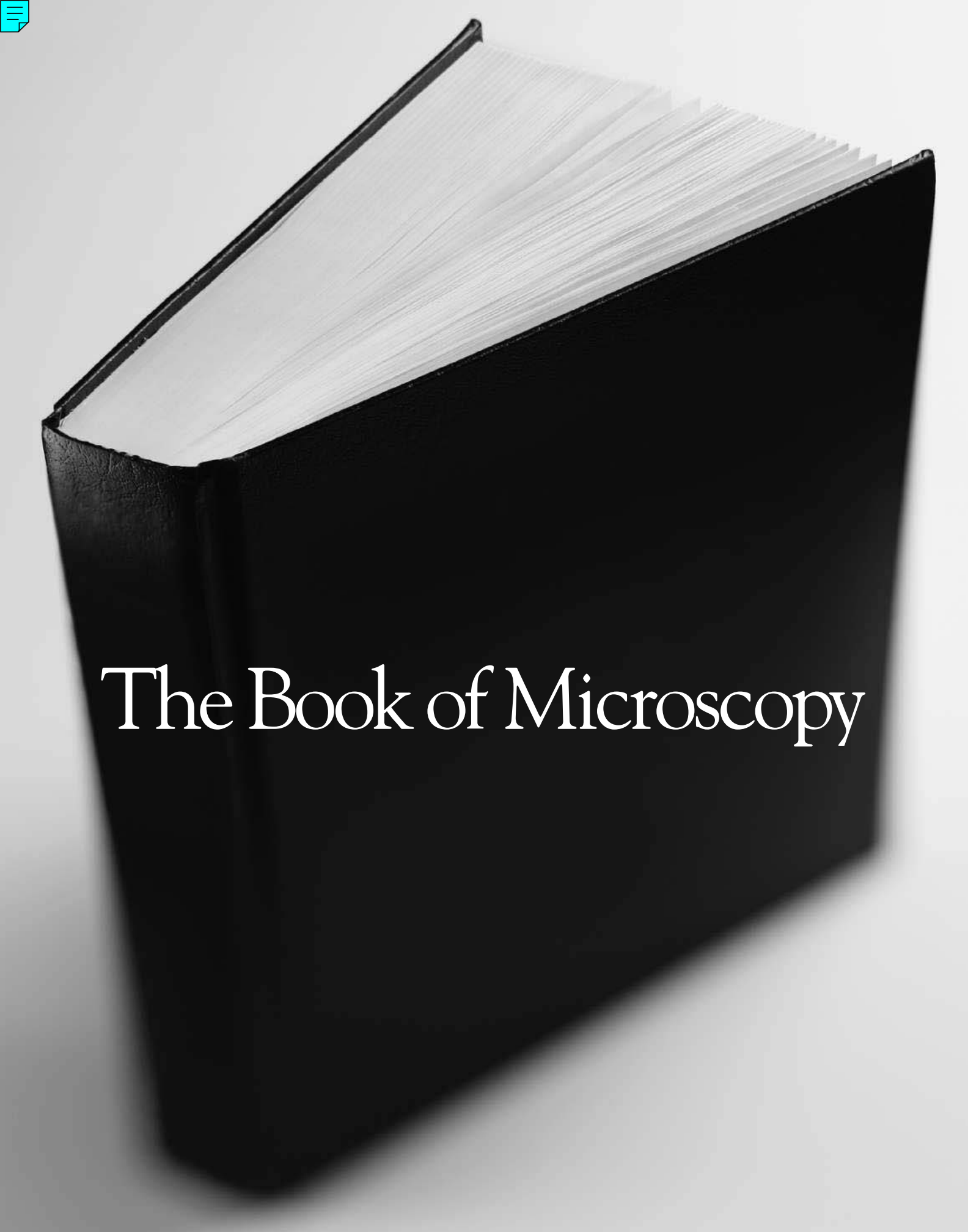
When Will the Oil Run Out?

IN HIS POLICY FORUM "OIL: NEVER CRY WOLF—why the petroleum age is far from over" (21 May 2004, p. 1114), L. Maugeri has done considerable double-counting to reassure us




The Book of Microscopy

that "the world is not running out of oil." He cites various upward revisions in historical estimates of oil reserves and resources, culminating in the 2000 U.S. Geological Survey estimate of 3021 billion barrels of ultimately recoverable resources, from which he draws the conclusion that "overall, the world retains more than 3 trillion barrels of recoverable oil resources."

Unfortunately, that 3021 figure includes the oil that has already been consumed. It consists of "undiscovered conventional oil" (732 billion barrels), "reserve growth" (688 billion barrels), "reserves remaining" (891 billion barrels), and "cumulative production" (710 billion barrels) (*1*). Deduct the 710 billion barrels already consumed, and the remaining oil totals 2311 billion barrels, a figure closer to other estimates.

Maugeri ignores the fact that the discovery of reserves lowers the estimate for undiscovered oil; it does not necessarily raise the total figure for ultimate recoverable oil resources.

He also calculates a "life index" of 40 years for known reserves, using current consumption figures. Using the 891 billion barrels (reserves remaining) figure would reduce that number to 32 years, but there are flaws in that calculation. Making projections based on current consumption is meaningless if consumption is rising (which it is). With a projected annual growth worldwide of 1.9% from 2001 to 2025 (*2*), the 32 years' supply would decline to about 26 years.

I believe we should draw very different conclusions from the present estimates. First, although the experts may quibble over their differences, they are in broad agreement: All the estimates I have seen agree within a factor of about two as to how much oil remains. The policy implications are not much different, wherever you may stand in that range. Oil resources are running down, and the supply is inelastic.

Second, we should be preparing now for a difficult transition. Europe and Japan, poor in oil resources, have gone much farther in making adjustments in energy use than the United States. Furthermore, they have stable or declining populations and similar requirements for energy and have chosen to limit demand, particularly by imposing high taxes on gasoline.

The United States is putting itself in the worst possible position for the energy transition by encouraging unchecked population growth (mostly driven by immigration) and doing very little to encourage energy efficiency.

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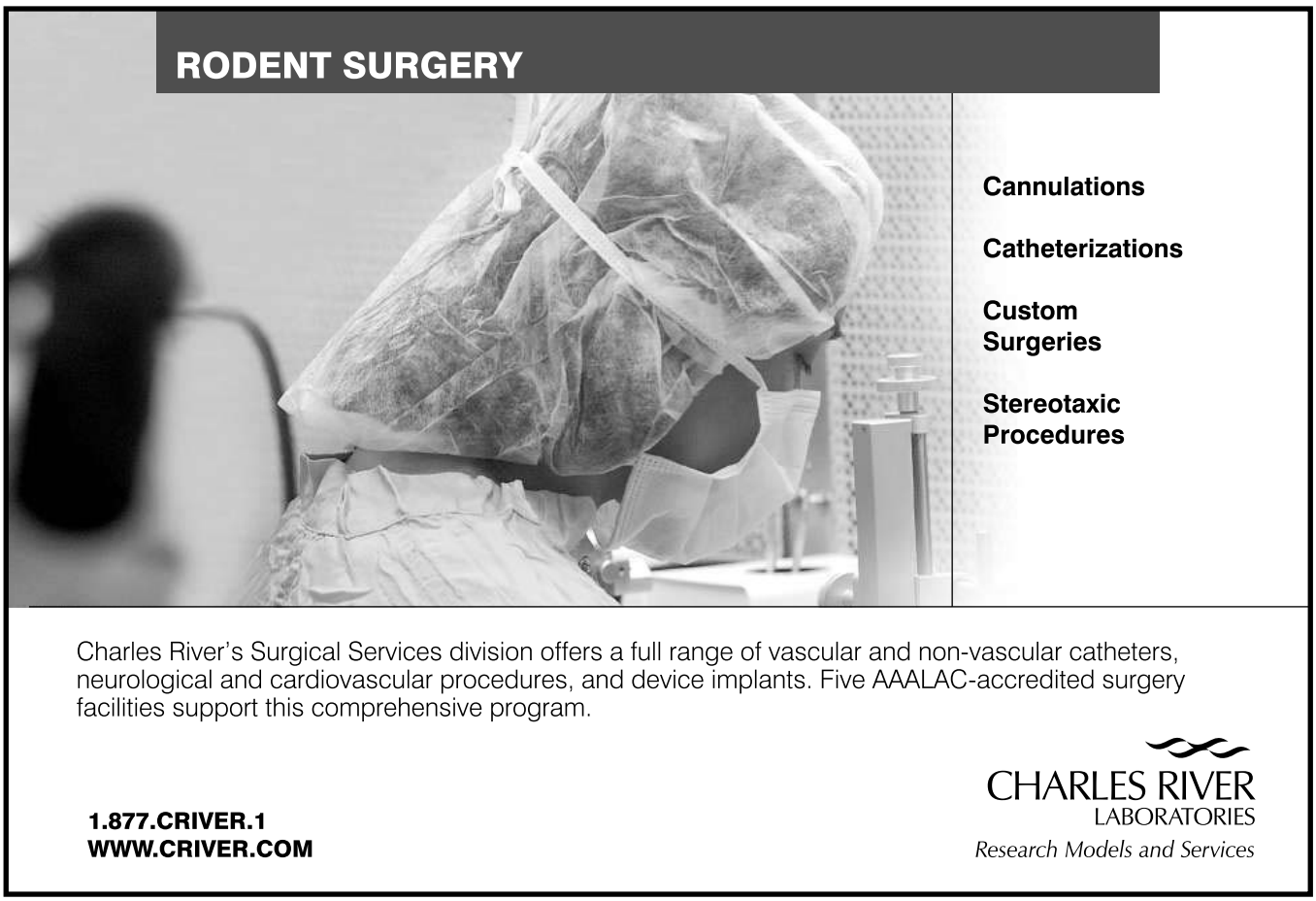
Recalculating Future Oil Reserves

IN HIS POLICY FORUM "OIL: NEVER CRY wolf—why the petroleum age is far from over" (21 May 2004, p. 1114), L. Maugeri claims that new discoveries of oil and other hydrocarbons will stave off oil scarcity for many generations to come. As the physicist Albert Bartlett (*1*) demonstrated nearly three decades ago, "When we are dealing with exponential growth we do not need to have an accurate estimate of the size of [the] resource in order to make a reliable estimate of how long the resource will last." Assume, he said, that the entire volume of Earth is oil (6.81×10^{21} barrels). At the then prevailing growth rate in oil consumption of 7.04%/year, "this earth full of oil [would] last only 342 years!" Now, with China and



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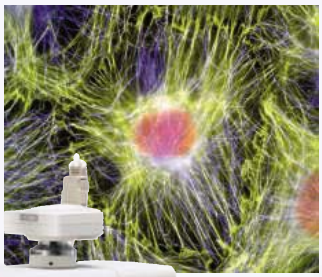

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other rapidly industrializing nations dramatically increasing their energy consumption, there seems little hope that exponential growth of hydrocarbon consumption will level off soon (*2*).

Of course, Earth is not made entirely of petroleum—far from it. Moreover, the alternative hydrocarbon sources that Maugeri mentions, Canadian tar sands and Venezuelan and Russian heavy oil, are no substitute for cheap oil. The petroleum geologist Walter Youngquist has noted that a considerable percentage of the energy recovered from these alternative sources is expended in their processing—two barrels out of every three in the case of tar sands and a similarly low net energy recovery for heavy oil (*3*). The same statement can be made about oil shale and biofuels. Ethanol from corn or sugar cane sometimes yields a net energy loss. The energy losses in producing and packaging hydrogen for the hydrogen economy will be considerable. Hydrogen is not a primary fuel, and its fundamental properties limit its ultimate utility. Nuclear power has a continuing role to play in generating electricity, but unlike oil, it is not a chemical feedstock, there are intractable safety concerns, and cheap oil and other hydrocarbons still mine and process the nuclear fuel and build the nuclear plants (*4*, *5*).

By referring to the legitimate concerns about oil scarcity as "hysteria" and "crying wolf," Maugeri deflects us from the only course that can save industrial civilization from the consequences of its overconsumption of energy. We need to begin a crash program to develop and implement energy-saving technologies in construction, manufacturing, transportation, and agriculture while the world still has enough oil wealth left to pay for the job. And at the same time, we have to speedily change a self-destructive mindset that glorifies waste and unnecessary consumption.

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CORRECTIONS AND CLARIFICATIONS

News of the Week: "Extrasolar planets get smaller and (possibly) harder" by R. Irion (17 June, p. 1727). The article incorrectly stated that a new planet circling the star Gliese 876 has the shortest exoplanet "year" yet seen, at 1.94 days. The Optical Gravitational Lensing Experiment (OGLE) has found three distant planets with shorter orbital periods. The fastest known is 1.21 days.

Random Samples: "Cetacean culture?" (10 June, p. 1545). Michael Krützen was identified as being at the University of Zurich. However, he did the work while at the University of New South Wales.

News Focus: "Structural genomics, round 2" by R. F. Service (11 Mar., p. 1554). Jeremy Berg was not identified. He is the director of the National Institute of General Medical Sciences, NIH. The definition given for a protein being "unique" was incorrect. The rule is that a unique protein structure must have less than 30% of its gene's sequence identical to the genetic sequence of any protein of known structure. The proper abbreviation for the Northeast Structural Genomics Consortium is NESG. Finally, rather than determining the manner in which an enzyme binds salicylic acid, NESG researchers discovered the manner in which the enzyme cleaves methyl salicylitate to produce salicylic acid.

TECHNICAL COMMENT ABSTRACTS

COMMENT ON "Children Creating Core Properties of Language: Evidence from an Emerging Sign Language in Nicaragua"

Tommaso Russo and Virginia Volterra

Senghas *et al.* (Reports, 17 Sept. 2004, p. 1779) presented Nicaraguan Sign Language as a language created by children who lacked exposure to a developed language. We stress the relevance of social and environmental factors in shaping language acquisition. The different communicative and linguistic inputs that elder and younger generations of Nicaraguans signers were exposed to could have influenced their task performance.
Full text at www.sciencemag.org/cgi/content/full/309/5731/56b

RESPONSE TO COMMENT ON "Children Creating Core Properties of Language: Evidence from an Emerging Sign Language in Nicaragua"

Ann Senghas, Asli Özyürek, Sotaro Kita

Russo and Volterra suggest that emergent structures were introduced to Nicaraguan Sign Language by Spanish-speakers through expressive channels such as writing, gesturing without speaking, or mouthing. However, these sources do not contain the relevant language structures, nor do their patterns of availability match the intergenerational differences observed. It is more likely that children's learning processes shaped the language.
Full text at www.sciencemag.org/cgi/content/full/309/5731/56c

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HISTORY OF SCIENCE

The Many Trials of Galileo Galilei

Peter Machamer

If you have ever wondered about Galileo and the myths that surround him, most of your questions will be answered in Maurice Finocchiaro's *Retrying Galileo, 1633–1992*. Was Galileo tortured? Were the actual charges against him about Copernicanism and the sun-centered universe or did they stem from his attempt to tell the Catholic Church how to interpret sacred scriptures? How has Galileo been portrayed as a martyr for free science in its contest with the authority of religion? How has the Church attempted over the years to defend its action of condemning Galileo as a heretic? These questions and many more are answered in the informative tales and fascinating stories that Finocchiaro (an emeritus professor of philosophy at the University of Nevada, Las Vegas) recounts. The author's discussions of Galileo's trial and its complex, 350-plus-year aftermath allow the reader to discover the amazingly diffuse ways and purposes that make up the history of the Galileo affair.

Retrying Galileo, 1633–1992
by Maurice Finocchiaro
University of California Press, Berkeley, CA, 2005. 497 pp. $50, £32.50. ISBN 0-520-24261-0.

Within the book you will encounter the saga of Napoleon, who after conquering part of Italy, in 1810 removed the Vatican archives to Paris and made plans to publish the original proceedings of Galileo's trial; following his fall in 1814, about two-thirds of the archives were destroyed or sold to cardboard manufacturers—some documents will never be recovered. Finocchiaro also recounts the intrigues and complex machinations of Giuseppe Settele's 1820 attempt to publish an astronomy textbook that treated Copernicanism and Earth's motion as a fact. The book's last story, set in 1992, tells of Pope John Paul II's sympathy for Galileo and yet how the commission he set up, under Cardinal Poupard, failed to "rehabilitate" Galileo. The author may, however, be a bit too charitable in his treatment of the attitudes of John Paul II (*1*).

Finocchiaro offers translations of a great number of historical documents that relate to the history of the Galileo affair, making the book a valuable resource. In his discussions of particular episodes, he typically starts by recounting some background and then presenting one or more important documents. He goes on to summarize and analyze these original reports in a nonjudgmental manner, pointing out how the positions taken compare to earlier assessments.

In a previous book (*2*), Finocchiaro published most of the documents relating to the trial itself, so few of these are presented in *Retrying Galileo*. The book begins with a description of the incidents surrounding the condemnation of Galileo in 1633. The author then moves on to tell how the news of the condemnation was promulgated and discussed. However, he withholds a surprise until a much later chapter that discusses the influential interpretation of Emil Wohlwill (1870), where he presents some important contemporary documents pertaining to Galileo's original trial. The bulk of the book lays out and analyzes reactions to and construals of the affair through the 17th, 18th, and 19th centuries. The five chapters devoted to developments in the 20th century portray the complex fate of Galileo in the hands of the modern church as well as his treatment by Bertolt Brecht and Arthur Koestler. From the many commentaries and allusions Finocchiaro discusses, it seems as though most everyone has had an opinion.

With a good book, it is always tempting to want more. But given the work's current length, such a demand would be foolish. Nonetheless, as I read the many translated documents I wondered about the social and political contexts to the events the author recounts. What was the background of the cardinals who presided over Galileo's 1633 trial? What role did the Thirty Years War play in bringing about the condemnation? What were Napoleon's intentions in closing the Vatican and moving its archives? Given that the 300th anniversary of Galileo's death fell during the height of World War II, what were the motivating factors when Pope Pius XII began, in 1941, an attempt to "rehabilitate" Galileo? But these matters fall outside the author's stated intent, and such regrets are mere cavils.

Some questions that arise might have been clarified further. For example, papal infallibility, as Finocchiaro mentions in passing, did not become dogma until the Vatican Council of 1870. Yet we read Jean D'Alembert, the physicist and natural philosopher, writing in a 1754 contribution to the famous *Encyclopédie* (which he co-edited with Denis Diderot) about those people, in Italy and not in enlightened France, who believe that the Pope is infallible. D'Alembert describes Galileo's condemnation as an "error so harmful to scientific progress" and attributes the error to this belief in infallibility. So, one wonders, what was the status and force of the doctrine of papal infallibility in the pre-1870 period?

At the end of *Retrying Galileo*, Finocchiaro surmises that the history of the Galileo affair is not over. Interestingly, in a speech delivered at Parma, Italy, 15 March 1990, then Cardinal Joseph Ratzinger (now Pope Benedict XVI) stated: "At the time of Galileo the Church remained much more faithful to reason than Galileo himself. The process against Galileo was reasonable and just" (*3*). Perhaps this portends the next story in the grand saga of the Galileo affair.

John Milton visiting Galileo. In his *Areopagitica* (1644), Milton cited Galileo's condemnation to support his arguments that freedom is essential to philosophy and that censorship will harm learning.

CREDIT: BETTMANN/CORBIS

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DANCE: PHYSICS

Dancing Einstein

John Bohannon

> I am satisfied with the mystery of life's eternity and with the awareness of—and glimpse into—the marvelous construction of the existing world together with the steadfast determination to comprehend a portion, be it ever so tiny, of the reason that manifests itself in nature. This is the basis of cosmic religiosity, and it appears to me that the most important function of art and science is to awaken this feeling among the receptive and keep it alive.
>
> —Albert Einstein (*1*)

It was with a tingling sense of dread that I entered a London theater last month, as if slouching toward the doctor's office with the expectation of bad news. To mark the 100th anniversary of Albert Einstein's most productive year of work, as well as the 50th year since his death, the Institute of Physics in London commissioned a young choreographer named Mark Baldwin to take Einstein's theories as inspiration for a dance. *Constant Speed* is the offspring of this marriage of science and art.

Mixing science with art seems like a great idea. Ever on the lookout for unexplored fields of human experience to mine, many artists see science as a mother lode. And scientists—exasperated by the blank stares that usually greet their enthusiastic statements, such as "Doesn't it just blow your mind how polypeptides fold their way through zillions of possible permutations in microseconds?"—cast their lonely eyes to artists to bridge the gap. So why is it that the result is so often either emotionally sterile (bad art) or intellectually superficial (bad science)? Perhaps, like science and religion, the two endeavors deal in mutually exclusive currency: science transmits knowledge while art transmits emotion, and mixing them inevitably dilutes the power and elegance of both.

To his credit, Baldwin was undaunted by such pessimism. "What I discovered," he says in the program notes, "is just how compatible dance and physics are." Indeed, just because an experiment is a long shot doesn't mean it's not worth trying.

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Hypothesis. Einstein's scientific theories carry emotional content that can be transmitted through dance.

Materials and methods. The experiment was performed within a sound- and light-insulated chamber of 1.5×10^4 cubic meters tapering to a stage. In a lowered pit before the stage, an orchestra performed a sequence of musical notes assembled in 1905 by the Viennese composer Franz Lehár. Simultaneously, the Rambert Dance Company (11 women and 11 men) executed a sequence of movements created this year by Mark Baldwin. Light—in wavelengths ranging from ultraviolet to deep red—was projected onto the dancers and reflected to a target surface. The target was a three-tiered array of chairs, each occupied (on a good night) by an observer. The observers were self-selected but were subject to the stringent filter of a £20 to £35 admission price. Relative to the light, sound waves from the orchestra pit reached observers with a delay that could be regarded as negligible for the purpose of the experiment. The warping of space-time between stationary observers and moving dancers could also be disregarded.

Constant Speed
Physics in Motion
Choreographed by Mark Baldwin

Rambert Dance Company, London. Sadler's Wells Theatre, London, 24–28 May 2005. For future performances, see www.rambert.org.uk

Results. Upon seeing the first sortie of dancers flit across the stage in head-to-toe, frilly white frocks, this observer mistook them for Woody Allen–esque spermatozoa. Although the dancers were adorably capricious, it was only with post hoc explanation that I realized that they represented pollen grains undergoing Brownian motion due to molecular collisions. (Einstein's 1905 model of the erratic jiggling of microscopic particles was used to prove the existence of atoms.)

CREDIT: TRISTRAM KENTON

After this uncertain start, the dance came into its own. With the arrival of a woman clad in undulating tutu and a blue puff ball sprouting from her crown—suggesting the dual nature of photons as particle and wave—we observers began to register rich emotional resonance. We giggled when a low-energy dancer had to be carried off stage by others in a pithy nod at the photoelectric effect. We sighed with delight through a sexy pas de deux between, it seemed, an atom and his swooning electron. And we gasped when one dancer, in a casual display of athletic virtuosity, flung himself into the air while spinning on two axes as if generating a personal electromagnetic field.

Zooming from the quantum to the galactic, we were treated to a playful exploration of relativity. Successfully translating the intuition-boggling fact that time flows faster or slower depending on one's perspective, the dancers often moved at tempos wildly different from that of the music. Other clever winks at the underlying theory included a roving duet between men in purple stalked by those in red or blue, inspired no doubt by the shift in light's wavelength as one moves toward or away from an object. But not all references hit their mark. When a giant mirrored ball descended from above—to underscore the quantum nature of light, according to Baldwin—I thought only of *Saturday Night Fever*.

During the single section of Lehár's score in a minor key, a solo dancer, alone on the stage like a nucleus in its immense cage of space, enacted the conversion of matter into energy. I was impressed that such pathos could be injected into something as abstract as $E = mc^2$, projecting the somber thought of an atomic bomb into our minds without uttering a word.

Repaying in kind, we speechless observers erupted at the end into thundering applause.

Conclusions. To test whether Einstein's theories had anything to do with the artistic success of Constant Speed, consider this Gedankenexperiment. We observers see the very same performance again, but without any knowledge of its subject matter. Will we enjoy it as much? Certainly not. I conclude with relief that science, in the hands of capable artists like Baldwin and the Rambert Dance Company, can be wrought into cultural expressions of sublime beauty. Einstein would have been proud.

Note

1. Einstein referred to his satisfaction with "the mystery of life's eternity" on several occasions. This quotation is from an essay broadcast on Edward R. Murrow's "This I Believe" series on CBS Radio in 1954, the year before Einstein's death.

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Ascent of Nanoscience in China

Chunli Bai

The three most widely used high-tech words in China now are "computer," "gene," and "nanometer," according to the China Association for Science and Technology. The ability to utter these words, of course, does not guarantee that the speaker understands their meanings and implications. I witnessed an episode that illustrates the point. A news reporter asked a woman he was interviewing for a story about nanotechnology if she had ever heard the term "nanometer." "Yes," the lady answered. But when the reporter asked her what she thought the word meant, the woman replied that it might denote a special kind of rice. She was in fact drawing upon her knowledge of the language. In Chinese, the word for "meter" has two meanings: One refers to the unit of length, and the other means rice. The woman's misunderstanding of the term "nanometer," in this case, is more amusing than concerning. But as nanoscience and nanotechnology become ever more consequential in our lives, we in the scientific community need to better inform and educate the public about the transformations this new nano era is likely to bring.

This yearlong essay series celebrates 125 years of *Science* by inviting researchers from around the world to provide a regional view of the scientific enterprise. Series editor, Ivan Amato

Along with its fast economic growth, China has embraced a national strategy for rejuvenating the country through education and science and technology. This strategy attaches importance to both fundamental research and the development of technologies that are critical to social and economic development. Among the fields that have enjoyed particularly rapid development in China in the past decade are nanoscience and nanotechnology. These terms refer to the growing knowledge base and technical framework for understanding and manipulating matter on nanometer scales ranging from the atomic to the cellular. Like many other countries, we in China expect that the development of nanoscience and nanotechnology will greatly affect many areas of scientific research and industrial development, and many aspects of everyday life. In time, we hope no one in China will think of rice when they hear the word "nanometer."

Nanoscience Takes Root

When the concept of nanoscience and nanotechnology was first introduced in the 1980s, it was received favorably in China. The initial interest was in part stimulated by the development of new tools and techniques for observing materials on the nanoscale, especially scanning probe microscopes (SPMs). Early explorations by Chinese scientists using scanning tunneling microscopes (STMs) and other types of SPMs helped build excitement about nanoscience and nanotechnology and led to visions of new techniques for revealing nanostructures and the novel properties that these structures can lead to.

Soon after the concept began trickling through the scientific ranks, the Chinese Academy of Sciences (CAS), the National Natural Science Foundation of China (NSFC), and the State Science and Technology Commission (SSTC) began funding nanoscience-related work and activities. Among the specific areas that received this early support were the development of scanning tunneling microscopy, then a groundbreaking technique for viewing the atomic and molecular landscapes of materials' surfaces, and nanomaterials research, in which investigators aim to engineer the optical, electronic, and other properties of materials by precisely controlling the structures' anatomy on the nanometer scale.

China also has helped those who work in nanoscience and nanotechnology to develop their sense of being part of a new research and development (R&D) community. Since 1990, for example, dozens of international and domestic conferences in the field have been held in China, including important early gatherings like the 7th International Conference on Scanning Tunneling Microscopy (1993) and the 4th International Conference on Nanometer-Scale Science and Technology (1996). These meetings, both held in Beijing, addressed a wide range of topics in nanoscience and nanotechnology and attracted wide attention and public interest.

In the 1990s, support for the development of nanoscience and nanotechnology increased substantially, largely through several major initiatives. In 1990, for example, SSTC launched the nearly decade-long "Climbing Up" project on nanomaterial science. In 1999,

CREDIT: CHUNLI BAI

Chunli Bai
China

Chunli Bai, executive vice president of the Chinese Academy of Sciences (CAS), shifted his research orientation from x-ray crystallography to the field of scanning tunneling microscopy while conducting his research work at Caltech as a visiting scholar in 1985. As the chief scientist of the China National Steering Committee for Nanoscience and Nanotechnology and the director of the China National Center for Nanoscience and Technology, he has been instrumental in furthering China's nanotechnology research both as a scientist and a policy-maker. He works with his colleagues and many of his graduate students on molecular nanostructures. He was elected a member of CAS and a Fellow of the Academy of Sciences for the Developing World (TWAS) in 1987. He is a recipient of the International Medal awarded by the Society of Chemical Industry (London-based) and delivered the TWAS 2002 Medal Lecture in Chemical Sciences. He has also won several awards and prizes conferred by the Chinese government and foundations and universities in Hong Kong.

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the Ministry of Science and Technology (MOST), whose predecessor was the SSTC, started a national basic research project entitled "Nanomaterial and Nanostructure" and has been funding basic research on nanomaterials, such as nanotubes, ever since. Our country's National High Technology Plan, which encompasses many categories of technology, has included a series of projects for nanomaterial applications. From 1990 to 2002 alone, nearly 1000 such projects (with a total funding of about $27 million) were implemented. In addition, during this period, NSFC approved nearly 1000 grants for small-scale projects in related areas. The scope of support was also greatly expanded to include specific areas such as nanodevices, nanobiology and medicine, detection and characterization, theory, modeling, and simulation.

With so much going on in nano-related R&D in so many different places in China, we created in 2000 the National Steering Committee for Nanoscience and Nanotechnology to oversee national policy and planning in these arenas. The committee was set up, among other organizations, by MOST, the State Development and Planning Commission, the Ministry of Education, CAS, the Chinese Academy of Engineering, and the NSFC.

Moving forward in nanoscience and nanotechnology requires a particularly wide spectrum of skills and knowledge. As such, a number of interdisciplinary research centers have been established to promote and facilitate collaborations between various institutions in a particular region by sharing of resources. The demand for multidisciplinary research platforms with components assembled from academia and industry and that also have educational functions has become especially strong in recent years. According to incomplete statistics, more than 50 universities, 20 institutes of CAS, and over 300 industry enterprises have engaged in nanoscience and nanotechnology R&D, with the involvement of more than 3000 researchers from different institutes, universities, and enterprises across China. The newly established National Center for Nanoscience and Technology in Beijing and the National Center for Nanoengineering in Shanghai are important additions to the list.

Operatic nanoscience. Now under construction in Beijing, China's new National Opera Hall features self-cleaning glass coated with a film of photocatalytic nanoparticles that can break down dirt.

CREDIT: LEI JIANG, INSTITUTE OF CHEMISTRY, CAS

Like many other countries, we in China expect that the development of nanoscience and nanotechnology will greatly affect many areas of scientific research and industrial development, and many aspects of everyday life.

Nanoscience Sends Out Branches

Of all the major topical areas of nanoscience and nanotechnology now being pursued by investigators in China, nanomaterials research has taken center stage.

A good representative of this fast-moving field is the family of nanomaterials known as carbon nanotubes (CNTs). These all-carbon tubes are just a few nanometers in diameter, which makes them comparable in girth to DNA molecules, and come in either single-walled varieties or multiwalled varieties with a nesting of carbon shells resembling the structure of a retractable antenna. The research group led by Sishen Xie at the Institute of Physics, one of CAS's many institutes in Beijing, invented a template-based growth method in 1996, by which both the diameter of multiwalled carbon nanotubes and the growth direction could be controlled. These features are important because they determine the properties and technological potential of these materials. In another development, a group led by Shoushan Fan at Tsinghua University made yarns out of carbon nanotubes. After appropriate heat treatment, these pure CNT yarns should eventually be able to be woven into a variety of macroscopic objects for different applications, such as bulletproof vests and materials that block electromagnetic waves.

Even traditional materials, such as copper, can be transformed when recast with nanoscience and nanotechnology in mind. A group led by Ke Lu at the Institute of Metal Research, yet another CAS institute, discovered in 2002 the superplastic property of nanostructured copper. The metal, with its nanoscale grains that are far finer than the grains of which standard copper is composed, can be elongated at room temperature to more than 50 times its original length without breaking. In 2004, Lu's group discovered another kind of nanocopper phenomenon, so-called copper growth twins, which is a specific type of crystalline microstructure. Copper with these nanoscale structural motifs has a tensile strength about 10 times as high as that of its conventional counterpart, while retaining electrical conductivity comparable to that of pure copper.

In the arena of inorganic materials, Dongyuan Zhao and his colleagues at Fudan University demonstrated a general synthetic strategy for creating stable multicomponent materials—such as mixed metal phosphates, mixed metal oxides, and metal borates—featuring a variety of porous structures. Such materials could lead to new families of catalysts, environmental filtration devices, and other technologies that rely on molecular interactions occurring in tiny nanoscale spaces. A morphological control approach was reported to selectively form SBA-15, a well-known silica-based material harboring a highly ordered hexagonal arrangement of nanoscale pores.

I, too, have been part of the nanoscience movement in China. My foray into this field began in 1985 when I first gained access to an ultrahigh-vacuum scanning tunneling microscope at the California Institute of Technology in the laboratory of John D. Baldeschwieler. I continued to work in the field after returning to CAS's Institute of Chemistry in Beijing, where I set up my own research group in 1987. With a homemade set of sophisticated tools—including a scanning tunneling microscope, an atomic force microscope (a variant of the STM), a ballistic electron emission microscope, and a scanning near-field optical microscope—my colleagues and I were able to join the nanoscience pioneers in China. In 1994, my group became known as the CAS Youth

Laboratory of Nanoscience and Nanotechnology and then in 2001 expanded to become the CAS Key Laboratory of Molecular Nanostructure and Nanotechnology. It now has more than 40 researchers and graduate students. With the return of several distinguished investigators from the United States, Japan, and Germany, the scope of research in the laboratory now includes the design and preparation of molecular nanostructures, novel nanomaterials, molecular nanodevices, single-molecule detection methods, and the development of techniques for characterizing nanoscale structures.

Even with all of this ongoing activity, the amount of support in China for nanoscience and nanotechnology is relatively small compared with that in the developed economies. In the United States, for example, an estimated $3 billion for nano-related research was earmarked by the government from 2001 to 2004 through the National Nanotechnology Initiative, a sum more than matched by venture capital. The figure for government funding in China now stands at about $160 million. Even so, the scientific output of Chinese nanoscientists is becoming ever more significant. According to the Scientific Citation Index, CAS ranked fourth in the world in total number of citations among those institutions and universities that published more than 100 nanotechnology papers from 1992 to 2002.* Another recent analysis of nanoscience productivity around the world ranked China at the top for the first 8 months of 2004.† This should not give the Chinese research community reason to be overly optimistic, however. The volume of published papers and total number of citations is only one indicator of the value of research. Another is the impact, or the number of citations per paper. From 2001 to 2003, the number of citations per nanotechnology paper published by scientists in the United States, Germany, Japan, and China was about 6.56, 4.54, 3.7, and 2.28, respectively, even though this figure for some Chinese research groups is much higher than the average. Another metric of innovation and activity in technological development is the number of patents awarded. As it turns out, the total number of patents acquired by China is far behind that of the developed economies. This is due both to a lack of technological innovation and to an insufficient amount of attention devoted to issues such as intellectual property protection.

To move forward and become more competitive in nanoscience and nanotechnology, China needs to continue to expand its now-limited research infrastructure. For example, there are too few nanoscale fabrication facilities for the role our country hopes to play in this new R&D arena. In some areas, such as nanoscale devices with novel electronic and optoelectronic features, efforts to consolidate resources to tackle key technological issues are under way. Efforts have also been made to pursue industrial-scale production of nanomaterials, such as carbon nanotubes, polymeric nanocomposites, and nanoparticle materials, with the intention of opening up opportunities for new businesses to sprout and grow.

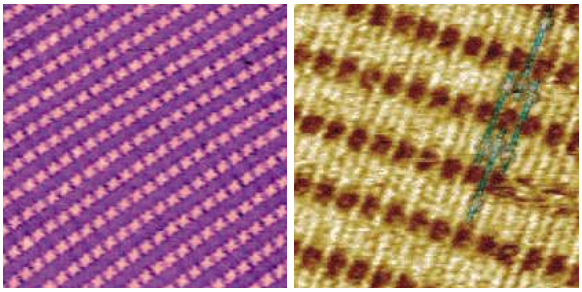
Because of the sudden popularity that the term "nano" enjoys, some firms in China have been finding that they can raise their profits simply by adding the label "nano" to their products.

Caveat Emptor

As China and many other countries embrace and develop nanoscience and nanotechnology, some are finding opportunities to exploit the novelty and public ignorance about these developments. Recall the woman who thought the word "nanoscience" referred to a kind of rice. Because of the sudden popularity that the term "nano" enjoys, some firms in China have been finding that they can raise their profits simply by adding the label "nano" to their products. We have heard of products such as nano-gas, nano-cups, nano-toothpaste, nano-beer, to name just a few. A few years ago, someone in Guangzhou in the south of China claimed that he had the know-how to produce nano-water. A few cups a day could prolong one's life, he claimed. He managed to fool a few investors, but he was ultimately exposed and punished by the local government.

The nano-water episode provides a cautionary tale for the regulatory communities and national and international standards organizations, which need to create and establish standardization and accreditation systems for nano products. In China, the effort to establish measurement standards for nanostuctures has resulted in the initiation of a national technical committee on nanotechnology standardization. Its work resulted this past February in the issuing of seven National Standardizations for Nanomaterials. Early in 2004, a special national committee was set up for laboratory accreditation under the auspices of the China National Board for Laboratories. This official body is charged with strengthening the inspection of research facilities in public institutions and with meeting the needs of manufacturers in China. These are necessary actions, given the imminent introduction of more and more commercial nano products into markets. The protection of public health also is of concern as the nanotechnology era unfolds. That is why safety assessment of nanomaterials, especially those intended for pharmaceutical use, is also being carefully carried out. As they are elsewhere in the world, toxicology studies are being conducted in a number of institutions, including CAS, Beijing University, and the Chinese Academy of Medical Sciences.

The nanoscience and nanotechnology community in China has made remarkable advances across the R&D spectrum, from fundamental scientific research to studies into the potential societal implications of new nanotechnologies. China still has a long way to go to improve the overall competitiveness of its nanoscience and nanotechnology enterprise, but all of the signs that I can see suggest it will become a leading contributor in the coming years.



Tiling with molecules. With his colleagues, Chunli Bai is exploiting molecules' own tendencies to self-organize. (**Left**) A scanning tunneling microscope (STM) image reveals porphyrin molecules that have arranged themselves on a graphite surface.‡ (**Right**) STM image shows metallacyclic molecules self-assembled on gold. The shape of one of these molecules is shown in a schematic (green). New types of catalysts, information-storage devices, and chemical sensors are among the potential applications of such self-assembled molecular structures.§

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NANOTECHNOLOGY

Small Things and Big Changes in the Developing World

Mohamed H. A. Hassan

Nanotechnology could prove to be a "transformative" technology comparable in its impact to the steam engine in the 18th century, electricity in the 20th century, and the Internet in contemporary society (*1*, *2*). Scientists are already developing nano-applications that are radically transforming a host of products and services, including battery-storage capacity, computer-chip minimization, drug delivery, facial creams, food processing, solar energy, and water purification (*3*, *4*).

The United States will spend an estimated US$3.7 billion on nanoscience and nanotechnology between now and 2008. Japan plans to spend more than US$3 billion during the same period. The European Commission authorized US$1.7 billion in its Sixth Framework Programme for Research and Technological Development (2002–2006), a figure that will likely rise to US$7.5 billion in its Seventh Framework Programme (2007–2013) (*5*–*7*). Clearly, those who have the resources have placed their bets on this next new thing.

The promise of nanoscience and nanotechnology. A paradigm shift is being embraced by developing countries as a way to meet social and economical goals.

Will such heavy investments lead to a North-South nanodivide comparable to the divide that has characterized biotechnology and global information technologies? The answer appears to be no.

In fact, nanoscience and nanotechnology may prove to be the first cutting-edge field to reflect the new realities of global science in the 21st century for two reasons. First, the capabilities and accomplishments of scientists and technologists from the developing world who choose to continue to work in their home countries are growing. Second, a number of governments in the South are devising ever more sophisticated and effective science and technology policies. On the downside, there is a disturbing emergence of a South-South gap in capabilities between scientifically proficient countries (Brazil, China, India, and Mexico, for example) and scientifically lagging countries, many of which are located in sub-Saharan Africa and in the Islamic world (*8*).

Between 2003 and 2007, China's central government will invest some US$240 million in nanoscience and nanotechnology and the nation's local governments, by some estimates, will provide US$360 million more. Brazil plans to invest more than US$25 million between 2004 and 2007, and India US$23 million between 2004 and 2009. Last year, South Africa invested an estimated US$6 million in this endeavor, and Argentina recently announced that that it will invest US$10 million over the next 5 years. Chile and Mexico are also pursuing modest but growing programs (*9*, *10*).

Smaller and poorer developing countries have also decided that this represents a strategic investment in future economic and social well-being that they cannot afford to ignore. Thailand and the Philippines, for example, are both devoting a portion of their small science and technology budgets to nanoscience and nanotechnology (*11*).

Such investments seem to be paying off. In 2004, scientists in China published more articles on nanoscience and nanotechnology in international peer-reviewed science journals than scientists in the United States, and it now ranks third behind the United States and Japan in nanotechnology patents. In a 2004 listing on nanotechnology and nanoscience literature, two developing countries (China and India) were among the top 10 nations in the publication of peer-reviewed articles (*12*).

What accounts for this dramatic change is the anticipated return on investment. The U.S. National Science Foundation (NSF) estimates that nanotechnology will represent a US$1 trillion global market by the end of this decade (*13*). That market would likely remain the sole domain of developed countries except for several interrelated factors.

First, the developing world's interest in nanoscience and nanotechnology parallels a trend in global science that has been unfolding over the past 20 years. This trend is highlighted by a number of developing countries that have embraced science and technology as critical elements in their overall economic development strategies.

China, for example, now devotes ~1.1% of its gross domestic product (GDP) to science and technology (it has recently become the world's third-largest investor in research and development in absolute terms). Meanwhile, the budget for China's National Natural Science Foundation (modeled on the NSF) has skyrocketed from US$10 million in 1986 to US$300 million in 2003. India, likewise, now invests ~1.2% of its GDP in science and technology and has emerged as one of the world's (not just the developing world's) leading countries in the application and, increasingly, the development of information technology (*14*). Brazil now spends an estimated ~1.1% of GDP on science and technology (*15*, *16*) and graduates some 7000 Ph.D. students in a broad range of scientific disciplines each year (*17*).

Second, the investment in nanoscience and nanotechnology represents a paradigm shift in science-based development strategies. As Turner T. Isoun, Nigeria's Federal Minister of Science and Technology, recently

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noted, "developing countries will not catch up with developed countries by investing in existing technologies alone. [In order] to compete successfully in global science today, a portion of the science and technology budget of every country must focus on cutting-edge science and technologies" (*18*).

This change in strategy explains, in part, China's extensive investments in biotechnology and information technologies. It explains the recent decision by the Brazilian parliament to allow stem cell research. It explains Nigeria's launch of a remote-sensing satellite in 2003 to improve resource management. And it explains South Africa's decision to become the chief sponsor of the Southern African Large Telescope, which will be the largest such instrument in the Southern hemisphere when it opens this autumn (*19*).

The participation of developing world scientists at the highest levels of research is likely to quicken the pace of global progress. Equally important, it is likely to help avoid a repetition of one concern that has hampered the development of genetically modified crops; namely, that monopolization of research and development by corporations in the United States has protected their commercial interests at the expense of the rest of the world (*20*).

If the development of nanoscience and nanotechnology becomes a truly global phenomenon and if scientists engage their fellow-citizens in an open dialogue on the fisks and benefits (*2*, *21*), then the public is likely to be more accepting.

Some nongovernmental organizations (NGOs) in the North, led by the Canadian-based ETC Group (*22*) have argued that nanotechnologies, by increasing the efficient use of raw materials and creating substitutes for them, could damage the commodities-dependent economies of developing countries. Although this could pose a problem in the short run, developing countries have no choice but to embrace nanoscience and nanotechnology if they hope to build successful economies in the long term.

As advances continue on a global scale, there are some ominous trends. Although increased investments in a number of developing countries have narrowed the North-South nanodivide, such investments have widened the South-South divide. Today, the environment for research and development in nanoscience and nanotechnology in Brazil, China, India, and South Africa bears closer resemblance to the research environment in Europe, Japan, and the United States than it does, for instance, to the research environment in the Dominican Republic, Laos, or Rwanda.

This is no small matter, for two reasons. First, having closer ties between scientists and technologists in the North and South increases the chances that the research and development agenda will be dictated by the North. Nanoscience and nanotechnology raise many intriguing questions from a research perspective. At the same time, they have many potentially valuable societal applications for poor people, including the creation of more efficient filtering systems for producing clean drinking water (through the creation of filters that prevent viruses and toxins from entering the water supply) and the provision of cheap and clean energy (through more efficient solar cells). But there remains the possibility that the majority of resources and expertise (in the North and South) may be applied to products and services that hold the most promising market potential in the North where the richest consumers live. To avoid this pitfall, governments throughout the developing world must focus on and support national policies that address critical social and environmental concerns in their own countries.

Specifically, the governments of those developing countries now investing heavily in nanotechnology should avoid "hitching" their research and development programs to those in the North. To prevent the creation of a South-South nanotechnology divide, such developing countries should devise broad-based strategies that include ample investments in South-South cooperation. In the long term, this could advance the use of these technologies worldwide and spur progress on many of the Millennium Development Goals (*23*).

It is for this reason that the global scientific community should pursue the following policies for the advancement of nanoscience and nanotechnology:

(i) establish nanotechnology centers of excellence in sub-Saharan Africa and other least-developed regions within existing competent institutions capable of partnering with other centers both in the South and North on joint projects;

(ii) forge networks between universities and research centers in scientifically proficient developing countries and universities and research centers in scientifically lagging countries, particularly those in the least developed countries;

(iii) develop national policies that explicitly call for investments in research projects that focus on issues of critical importance in the developing world, including access to safe drinking water; the development of low-cost yet efficient sources of renewable energy; and the creation of gels that can reduce the risk of HIV/AIDS transmission.

Proponents of nanoscience and nanotechnology claim that this transformative field could radically alter fundamental aspects of our global society. If the research is organized in an effective manner, the pursuit of these larger social and environmental goals could also help build the capacities of nations that have been excluded. Of all the benefits promised by nanoscience and nanotechnology, the potential to lift the quality of science and technology on a global scale may be the most important benefit of all.

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MATERIALS SCIENCE

Expanding the Molecular Electronics Toolbox

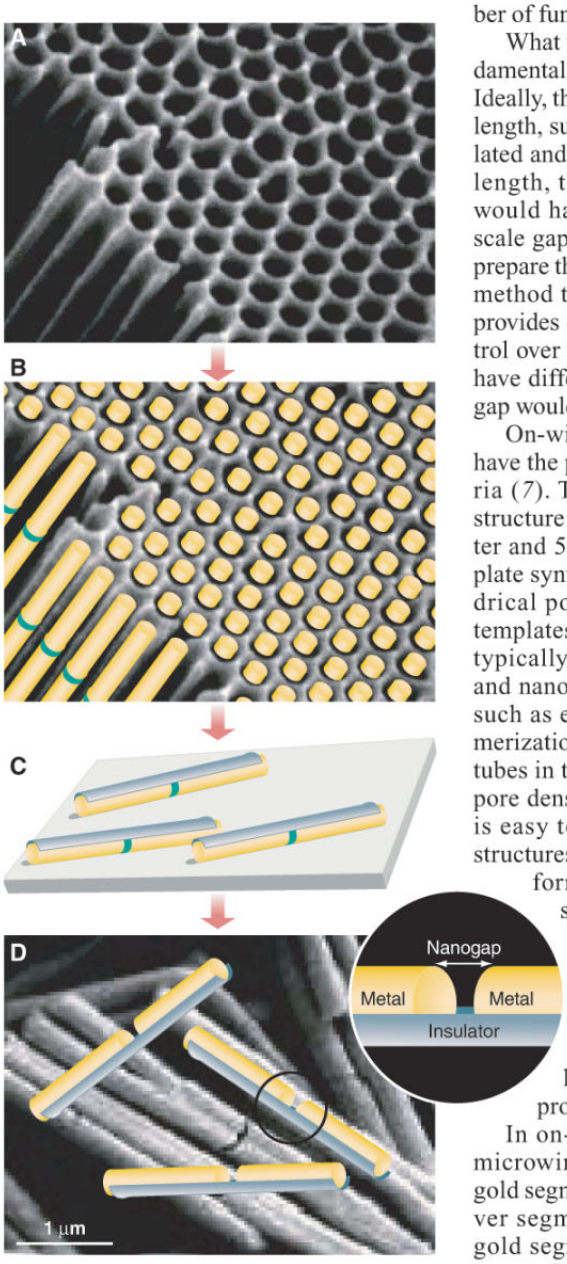
Charles R. Martin and Lane A. Baker

The goal of molecular electronics is the construction of electronic circuit elements (such as transistors and diodes) from individual molecules (*1*, *2*). The molecules of interest have dimensions on the order of a few nanometers, whereas with conventional photolithography, the smallest structures that can be prepared are on the order of 100 nm (*3*). Therefore, molecular electronics potentially allows a greater number of circuit elements to be packed on a chip than is possible with conventional methods (*4*). Mainstream electronics companies such as Hewlett-Packard, Motorola, and IBM are pursuing research and development projects in molecular electronics (*5*).

However, the development of this technology requires electrical contact to the molecule to be made (*1*, *6*). To study the electronic properties of a macroscopic circuit element, such as a resistor, one simply connects electrical leads to each end of the device. How can one connect electrical leads to each end of a molecule? On page 113 of this issue, Qin *et al.* describe a potentially versatile method, on-wire lithography, for accomplishing this task (*7*).

Currently, two general approaches are used to make electrical contacts to individual molecules (*1*). In the first approach, one end of the molecule is connected to a conductive surface, and the ultrafine tip of a scanning-probe microscope is used to make contact to another part of the molecule. However, it can be difficult to find a single molecule on a surface and to know how much force to apply to make good electrical contact (*6*).

The second approach entails fabrication of a nanometer-scale gap between two electrodes, followed by insertion of the molecule into the gap (*1*). In one such method, a gold nanowire is prepared that is thick at its ends but thin in the middle (*8*). When an electrical current passes through this wire, the thin part of the wire breaks to create the gap. In an alternative strategy, a thin metallic bridge is created across a pit in a silicon wafer (*9*). The wafer is then strained in order to break the bridge at the thinnest point along its length. Such methods typically require experimental finesse and sophisticated modern microfabrication facilities, and often yield only a small number of functional devices (*8*).



On-wire lithography. A nanopore membrane (**A**) is used as the template for synthesizing segmented nanowires (**B**). Next, the template is dissolved, the nanowires are deposited on a surface, and silicon is deposited selectively onto segmented nanowires (**C**). Removal of sacrificial metal (green) creates a nanometer-scale gap between the two conducting (gold) segments (**D**).

What would be the ideal device for fundamental studies in molecular electronics? Ideally, the device would be micrometers in length, such that it could be easily manipulated and positioned. Somewhere along its length, this micrometer-scale structure would have a reproducible, nanometer-scale gap. Finally, it should be possible to prepare the device by a simple and versatile method that allows mass production and provides convenient and reproducible control over the size of the gap. The ability to have different metals on either side of the gap would also be useful (*10*).

On-wire lithography yields devices that have the potential to satisfy all these criteria (*7*). The requisite micrometer-scale structure is a microwire (360 nm in diameter and 5 μm in length) prepared by template synthesis (*11*). In this method, cylindrical pores in a membrane are used as templates to prepare wires and tubes that typically have micrometer-scale lengths and nanometer-scale diameters. Methods such as electroplating or chemical polymerization are used to deposit the wires or tubes in the pores. Because the membrane pore densities are high (see the figure), it is easy to make large numbers of these structures. In addition, the pores have uniform diameters, resulting in correspondingly uniform tubes and wires, and the pore diameter can be varied at will. After preparation, the wires or tubes can be liberated by dissolving the template membrane, and manipulated by simple solution-based processing methods.

In on-wire lithography, a segmented microwire—in the simplest case, a long gold segment connected to a very short silver segment connected to another long gold segment—is electroplated into the pores of a commercially available alumina template. Template-based electroplating of such segmented wires is well established (*12*, *13*), and electronic measurements on segmented nanowires have been reported (*14*). The gap is prepared by dissolving the short silver segment. Electroplating provides a reproducible and

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quantifiable way to control the quantity of material deposited. Hence, the length of the gap-forming segment can be controlled with great precision. Furthermore, it is easy to prepare wires with more than one gap and with gaps of different lengths.

It would be pointless to have a dissolvable gap between two nondissolvable segments if, upon dissolution, the remaining segments fell apart. Qin *et al.* solve this problem by dispersing the segmented wires on a surface and then coating one side with a sheath of a nondissolvable material. This sheath holds the wire together after removal of the dissolvable segment. The authors show that these sheathed microwires can be placed between two much larger electrodes and that the gap can be bridged with a test molecular-electronic material—a conductive polymer.

Challenges remain. The smallest gap reported by Qin *et al.* is 5 nm. There is no fundamental reason why smaller gaps cannot be prepared, but this remains to be demonstrated. Furthermore, the test material consisted of a collection of polymeric chains. The method has yet be demonstrated on a single small-molecule conductor. Nevertheless, the ease with which the devices can be prepared, characterized, and manipulated bodes well for on-wire lithography to become an important tool in the molecular-electronics toolbox.

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ECOLOGY

Food Web Ecology: Playing Jenga and Beyond

Peter C. de Ruiter, Volkmar Wolters, John C. Moore, Kirk O. Winemiller

Naturalists have long noted that the distribution, abundance, and behavior of organisms are influenced by interactions with other species (*1*). Motivated in part by Paine's (*2*) work in the rocky intertidal zone and May's (*3*) theoretical work on the relationship between the complexity and the stability of ecosystems, the study of food webs gained momentum in the late 1970s and early 1980s (*4*). These studies precipitated a convergence of different approaches—mathematical treatments, descriptive work, manipulative field studies, and a formal treatment of energy flow and matter. This in turn allowed mapping of the interrelationships among the structure of an ecological community, its stability, and the processes occurring within the ecosystem—that is, construction of a food web (*5*).

Over the past decade, new issues arising in ecology, such as environmental change, spatial ecology, and the functional implications of biodiversity, require a different view of ecosystems and ecological research (*6*). The food web approach, with its focus on static structure and reliance on stability or persistence of species, seemed ill-equipped for analyzing these more dynamic topics.

Indeed, the often-used metaphor for the relationship among species, community structure, and stability was that of a stone arch with the loading forces among stones (species) representing interactions among species, and the "keystone" representing the species that had the dominant role in regulating structure and stability of the community. But many ecologists now view such a static representation of biological communities as inappropriate. Moreover, food web descriptions have been criticized for incompleteness because they do not fully account for all the species and links that are present, and because they generally ignore spatial and temporal variability. For these reasons, food web approaches have rarely been applied to current environmental issues.

Jenga. In a game of Jenga, players successively take away parts and place them on top until the structure becomes unstable and crashes. Each part can thus be a keystone. When parts are replaced at other positions, the stability of the Jenga structure can be maintained.

The metaphor of the static arch might better be replaced with the metaphor of the structures built during a variation of the game Jenga (see the first figure, caption). Simple rules of balance and energetics govern the stability of both arch and Jenga structures, but unlike an arch, a Jenga structure is constantly changing, with additions and deletions of stones, and its stability at any moment depends on the importance of a given ingoing or outgoing stone's contribution to the structure. By realizing that dynamics are key to understanding complex structures, we can see stable food webs not as static entities, but as open and flexible Jenga-like systems that can change in species attributes, composition, and dynamics. Recent food web studies have incorporated data on spatial and temporal dynamics. Here, we highlight a few examples of such studies and discuss their implications for environmental management.

Over time, food webs change in species composition and in population life history parameters and abundances, and individual organisms within the web change in growth, size, and behavior. Dynamic relationships among different levels of the biological hierarchy govern food web structure and stability. Field observations and theoretical models show that environmental heterogeneity creates subsystems

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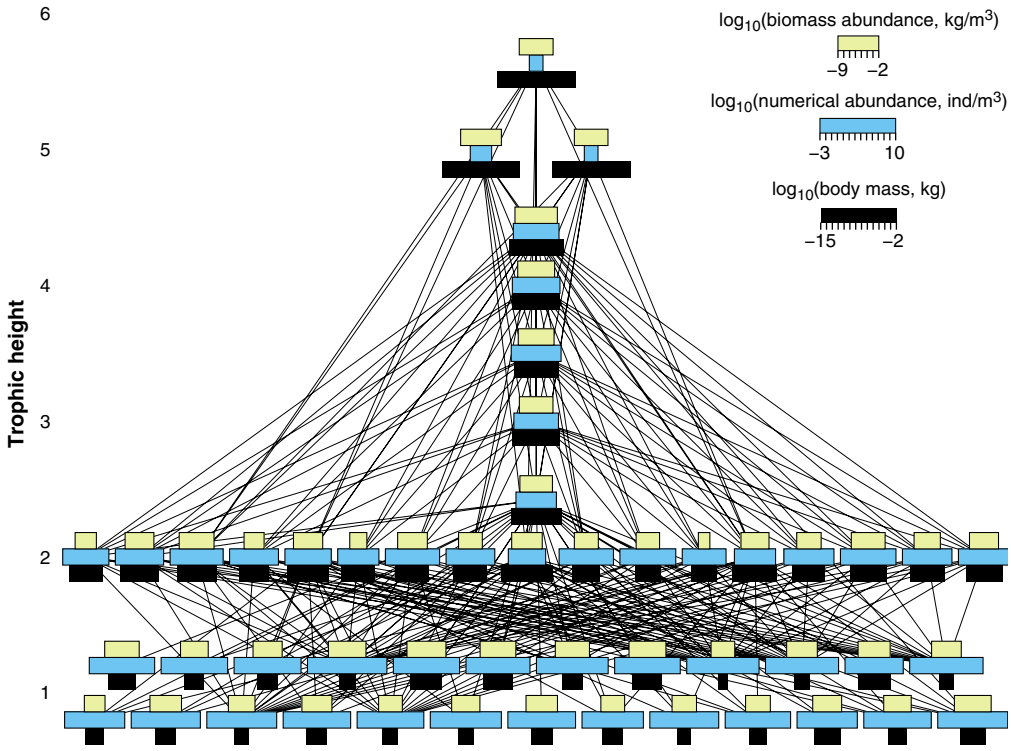

The food web of Tuesday Lake, 1984. The width of the horizontal bars shows the body mass ($\log_{10}$ kg), number ($\log_{10}$ individuals per m^3), and biomass ($\log_{10}$ kg/m^3), respectively, of each species. The vertical positions of the species show trophic height (*20*). Despite a major change in species composition, following a manipulation, this energetic setup of the food web remained roughly the same (*19*).

(compartments) of interacting species within food webs, especially at the lower trophic levels (*7*, *8*). Organisms at the higher trophic levels act as integrators, linking the lower pathways in space and time, and stabilize the dynamics of their resources (prey) via density-dependent foraging (*9*, *10*). This relationship explicitly supports MacArthur's idea (*1*) that community complexity buffers against perturbations and thereby overrides the inherent constraints on system stability imposed by complexity (*3*). Remarkably, this mechanism is similar to the way in which dynamics at the level of individual behavior influence food web structure and stability. For example, a predator switching to new prey affects population dynamics, because such dietary shifts inhibit rapid population growth of abundant prey while allowing rare prey to increase (*11*). Such shifts can be rapid; hence, when food web architecture changes (by changes in species composition or through fluctuating population abundances), web structure may quickly stabilize and may even result in a positive complexity-stability relationship.

Life history processes in structured populations also can influence community dynamics in extraordinary and even counterintuitive ways. Size-structured food web models, in which growth in body size is density-dependent, predict that size-selective predators decrease the overall biomass of the prey as expected, but change the prey size distribution. This alters competition among prey individuals to such an extent that it actually leads to an increase in the abundance of the preferred prey stages. This positive feedback allows for large populations of predators to persist under conditions where small populations are likely to become extinct (*12*).

The trophic position of species in dynamic food webs may influence the risk of loss of that species, with possible consequences for ecosystem functioning (*13*). Experiments on pond food webs show that the effects of species on ecosystem processes depend on the interplay between environmental factors (such as productivity) and trophic position, whereby species at higher trophic levels tend to have larger effects (*14*). The risk of a given species' extinction and its consequences (in terms of secondary extinctions and ecosystem functioning) will be different in different ecosystems and will vary within ecosystems over space and time. Hence, a keystone species in one setting may have relatively little influence on community dynamics in another setting.

What does this research tell us? First, the performance and persistence of species and the role of species in biological communities should be examined in the context of a dynamic food web. The studies also show how to go beyond playing Jenga by revealing the general rules of demography and energetics that tend to stabilize biological communities (*15*, *16*). The stabilizing effect of flexible substructures imposed by environmental change and variability (*9*) echoes the notion of stabilizing trophic pyramids (*17*) occurring in food webs and food web compartments (*10*). When compartments are viewed as trophic interaction loops, we can even understand mathematically the stabilizing effect of pyramidal structures within loops on food web structure (*18*).

Dynamics and stability can be seen in the intensively studied food web of Tuesday Lake in Michigan, USA (see the second figure). Removal of three planktivorous fish species and addition of one piscivorous fish species changed the lake's community structure remarkably. Although this manipulation had almost no effect on species richness (56 in 1984, 57 in 1986), about 50% of the species were replaced by new incoming species within less than 2 years (*19*). The energetic setup of the food web, in terms of distributions of body sizes and abundances over trophic levels, unexpectedly remained roughly the same. Apparently, the new web structure allowed the community to conserve key ecological features in the face of a major disturbance.

The notion of the ecosystem as a static arch has restricted our vision. In contrast, viewing food webs as open and flexible Jenga-like structures that accommodate changes in species composition, attributes, and dynamics reveals the features of the ecosystem that are critical to our understanding of community resistance and resilience to environmental change and disturbance. Recent theoretical advances in food web research must be accompanied by rigorous experiments and detailed empirical studies of food web modules in a variety of ecosystems. As food web science continues to develop, it surely will contribute new tools and new perspectives for the management of both natural and human-affected ecosystems.

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ASTRONOMY

Masers in the Sky

Moshe Elitzur

In 1963, radio emission from interstellar OH (the hydroxyl radical) was discovered. The emission patterns in the astronomical sources deviated considerably from expectations based on laboratory conditions. Two years later, researchers realized that some of the most peculiar emission properties of interstellar OH could only be explained in terms of maser amplification. (Maser is an acronym for microwave amplification by stimulated emission of radiation; masers operate on the same principles as lasers, except that they involve microwave radiation instead of visible light.)

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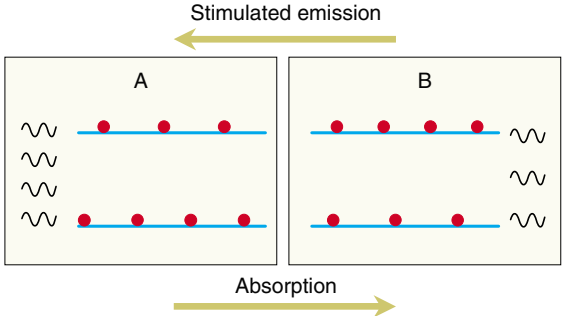
Maser emission has now been detected from many different molecules in a variety of astronomical sources, from nearby comets to faraway galaxies. But the evidence for amplification is indirect in most cases. Observations from 18 pulsars, reported by Weisberg *et al.* on page 106 of this issue (*1*), provide direct evidence for an interstellar amplifier in the direction of one of these pulsars, B1641-45.

Every 0.455 seconds, B1641-45 emits a pulse of radio radiation toward Earth that passes through an OH cloud. Spectra of the four OH ground-state lines detected from the cloud display absorption features in three lines and an emission feature in the fourth [see figure 3 in (*1*)]. When the pulsar is on, passage of its radiation through the cloud deepens the absorption features, just like the shadow cast by an object in front of a bright light. But the emission feature in the fourth line becomes stronger after passing through the intervening screen, the equivalent of an object amplifying a background light instead of casting a shadow.

Almost 30 years ago, Rieu *et al.* observed a similar effect for an OH cloud in front of a distant radio galaxy when they switched the telescope between pointing directly at the source and pointing away from it (*2*). In both cases (*1*, *2*), the input signals are amplified by only a few percent, but to date, these weak masers are the only unambiguous direct evidence for amplification. The pulsar method (*1*) increases confidence in the results, thanks to the repeated detection of signals that arrive more than twice every second.

The maser effect clearly occurs easily in interstellar space, even though it requires special effort on Earth. This disparity reflects fundamental requisites for laser and maser operation. Consider two states, A and B, of a system containing electromagnetic radiation (such as light or radio emission) and microscopic particles (such as atoms or molecules) (see the figure). The total energy is the same in both states; A contains an extra photon, whereas in B, one particle has moved from the lower to the upper level. The process that takes the system from A to B is called absorption and is the reason why the intensity of radiation is attenuated when it passes through matter. The process that takes the system from B to A is called stimulated emission—the interaction of radiation with particles leads to the emission of an additional photon. This process is the essence of the maser effect. As the reverse of absorption, stimulated emission amplifies the radiation.



Absorption versus stimulated emission. Sketched are two states, A and B. Each state has two energy levels, with some particles populating each level. The frequency of the radiation (represented by waves) is matched to the energy separation between the levels, such that it can interact with the particles. The number of waves represents the radiation intensity, that is, the number of photons.

In any given source, the net balance of absorptions and stimulated emissions depends on the particle population distribution between the two levels. Under most circumstances, this distribution follows the rules of thermodynamic equilibrium, and the population of the upper level is so small that stimulated emissions can be ignored; the material attenuates radiation. But when the number of particles in the upper level exceeds that in the lower level, a situation called population inversion, stimulated emissions outnumber absorptions and the radiation is amplified. Such an extreme distribution can occur only at low matter densities, and interstellar space thus provides an ideal setting; its density is so low that its densest regions are comparable to the best laboratory vacuum. Thus, thermodynamic equilibrium is the rule in terrestrial circumstances but is the exception in interstellar space, making the latter a natural environment for maser operation.

Maser radiation can be extremely bright; the temperature equivalent of brightness sometimes exceeds 10^{15} K. These intense beacons enable radio imaging with an angular resolution of 0.0003 arc sec; if the human eye had this resolving power, these words could be read from a distance of ~3000 miles. Separations between neighboring maser spots are measured with the even higher accuracy of 0.00001 arc sec.

It was thanks to this resolving power that the existence of supermassive black holes at the center of galaxies was established. Miyoshi *et al.* (*3*) found that the galaxy NGC 4258 harbors a disk-like structure, with masers of water vapor revolving around the center just like the planets revolve around the Sun. From the orbital rotations, the authors determined that a central mass 7×10^7 times that of the Sun is contained in a region no larger than the solar system. No object other than a black hole can have such a high mass density.

Amplified radiation has distinct properties markedly different from those of ordi-

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nary radiation. As the pulsar observations of Weisberg *et al.* (*1*) demonstrate, even weak amplification can produce astounding effects. Comets provide another example. When a comet approaches the Sun, the rising rate of heating causes ice evaporation and the production of OH, whose population distribution is controlled by interaction with solar ultraviolet radiation. Because the heliocentric velocity of the comet varies during its motion around the Sun, the Doppler effect shifts different solar lines into and out of match with the OH transition frequencies. At some locations the net outcome is population inversion, at others the opposite. As a result, the detected OH lines can oscillate between emission and absorption as the comet moves around the Sun. Since 1973, these striking oscillations have been observed in many comets (*4*).

Weak amplification can even offer advantages. Weak OH maser emission at 1720 MHz is always accompanied by absorption at the 1612 MHz transition, and the sum of these conjugate features is zero. When such cancellation is observed, the two detected features must originate from the same region, removing a critical uncertainty for sources at cosmological distances (distances of billions of light years). This approach has recently been used to place limits on the possible variation of fundamental constants, such as the electron-proton mass ratio, during the evolution of our universe (*5*).

As demonstrated by galaxy NGC 4258, maser radiation can provide unique information about small details in the structure of the emitting astronomical sources. Strong maser radiation is emitted during both the very early and very late stages of the life of a star, providing invaluable information on stellar evolution. For example, recent analysis of methanol maser observations established the existence of a circumstellar disk around a newly formed star (*6*). The disk structure is extremely smooth, providing a glimpse of the state of our own solar system before the planets condensed out of its protoplanetary disk.

To fully understand the structure of an astronomical source, one needs data at different regions of the electromagnetic spectrum. Thanks to masers, data at radio wavelengths are much more detailed than at any other wavelength. Facilities are under construction and in the planning stages to extend high-resolution astronomy from radio to infrared and even visible wavelengths. As these regions of the electromagnetic spectrum are combined with maser observations, we will gain valuable insight into the detailed structure and inner workings of a wide range of astronomical objects.

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GENETICS

Themes and Variations in Apicomplexan Parasite Biology

David S. Roos

The eukaryotic phylum Apicomplexa comprises more than 5000 species of parasitic protozoa (*1*), including the *Plasmodium* parasites responsible for malaria. *Toxoplasma gondii* is well known as a source of congenital neurological birth defects, while *Cryptosporidium* and *Cyclospora* (along with *Toxoplasma*) have emerged as opportunistic infections associated with immunosuppressive conditions (including AIDS), and as sources of human infection through contaminated food or water supplies. Many apicomplexan parasites are also of veterinary importance, including *Babesia*, *Eimeria*, *Neospora*, *Sarcocystis*, and *Theileria*. *Theileria parva* and *T. annulata* are cattle pathogens, responsible for East Coast fever and theileriosis. Acute lymphoproliferative disease or anemia can lead to death, imposing significant constraints on cattle-farming in sub-Saharan Africa (*2*).

Theileria parasites are also of considerable biological interest, as the only eukaryotic pathogens known to transform lymphocytes (*3*). Parasite sporozoites invade lymphocytes, escape from the invasion vacuole, interact with the host cell cytoskeleton (*4*), and alter cellular signaling pathways (*5*) through mechanisms that are incompletely understood. Further insight into their fascinating biology comes from two reports in this issue, by Gardner *et al.* on page 134 (*6*) and Pain *et al.* on page 131 (*7*), that describe effectively complete genome sequences for *T. parva* and *T. annulata*, respectively.

The availability of two *Theileria* genomes, along with numerous sequences for other apicomplexans (*8*–*13*), provides a rich trove of data for comparative analysis (see the Table) (*14*). Consistent with observations in other parasites, the *Theileria* genome is reduced in both metabolic complexity and size (~4000 genes, 8.4 Mb) relative to the genomes of other eukaryotes. Absent genes suggest metabolic deficiencies in the synthesis of purines, polyamines, fatty acids, and porphyrin, among other pathways (*6*). The parasites are able to carry out glycolysis, and probably the tricarboxylic acid cycle, although how these pathways are linked is unclear. Moderate levels of synteny are observed between *Theileria* and *Plasmodium* genomes (*6*).

Apicomplexan parasites pursue diverse life-history strategies, infecting virtually all animals, from mollusks to mammals (*1*). Some parasite life cycles are relatively simple, involving only a single host (see the figure), whereas others require sexual recombination in a vector species for transmission. Some parasites are specialists, restricted to particular species and tissues, whereas others are generalists. For example, *Plasmodium falciparum*, which causes the most lethal form of malaria, infects only great apes (including humans), and is transmitted only by anopheline mosquitoes. In contrast, *T. gondii* can infect almost any tissue of warm-blooded animals, causing disease in immunodeficient hosts (including AIDS patients and human and animal fetuses).

The complex life-cycle stages of apicomplexan parasite infection are characterized by persistent themes, with subtle variations. Extracellular "zoite" forms are usually motile, and include an "apical complex" that gives the phylum its name, including organelles associated with host cell attachment, invasion, and establishment of an intracellular "parasitophorous vacuole" (*15*). *Theileria* sporozoites and merozoites are unusual in being nonmotile, and appear to invade host cells passively, in an orientation-independent manner (*4*). Consistent with these observations, organelles usually found in the apical complex are modified: The distinctive cytoskeletal "conoid" (*16*) is absent; the micronemes (*17*), whose secretion is associated with cell adhesion, are altered or absent; and the apicoplast (*18*, *19*), a secondary endosymbiotic organelle that may play a role in establishing the parasitophorous vacuole, shows reduced function. *Theileria* has retained the rhoptries, however; these secretory organelles (*17*) are also part of the apical complex, and are suspected to function in modifying the parasite's intracellular

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COMPARATIVE BIOLOGY OF THE APICOMPLEXA

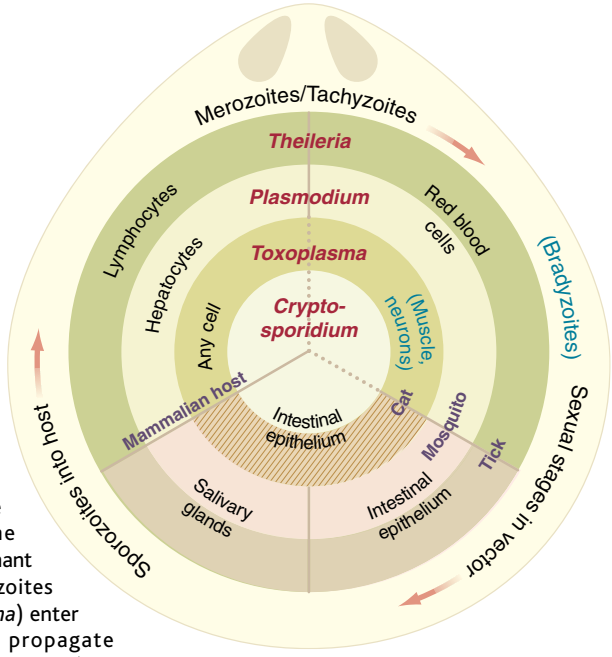
Genus (genome size; no. available)	Transmission vector (definitive host)	Apical organelles* Co	Mn	Rh	Ap	Intracellular compartment	Distinctive biology	Function of expanded gene families
Cryptosporidium (9 Mb, two genomes)	None required	+	+	+	–	Feeder organelle	Extreme metabolic reduction, and nutrient salvage from host	Surface antigens (mucins) Transporters
Toxoplasma (65 Mb, one genome)	Cats (not required for asexual transmission)	+	+	+	+	Parasitophorous vacuole	Long-term persistence in brain and other tissues	Parasite surface antigens Nutrient salvage?
Plasmodium (23 Mb, six + genomes)	Mosquitoes	–	+	+	+	Parasitophorous vacuole	Modification of infected RBCs mediates cytoadherence/sequestration	RBC surface proteins Nutrient salvage?
Theileria (8 Mb, two genomes)	Ticks	–	(–)	+	(+)	Cytoplasm	Lymphocyte transformation	Parasite surface antigens Lymphocyte transformation?

*Co, conoid; Mn, micronemes; Rh, rhoptries; Ap, apicoplast. Parentheses reflect the absence of micronemes in at least some zoite stages and reduced metabolic function of the apicoplast (*6*).

home for survival (*20*). In the case of *Theileria*, rhoptry secretion coincides with lysis of the invasion compartment, releasing parasites into the host cell cytoplasm (*3*, *4*).

In addition to the insights that they provide into basic metabolic pathways, genome sequences also highlight phylogenetically restricted genes, which are often linked to distinctive aspects of organismal biology. Large families of surface antigens are commonly found in pathogen genomes, and are likely to play an important role in antigenic variation and immune evasion (*12*–*14*, *21*, *22*). *Plasmodium* genomes even encode proteins targeted into the infected red blood cell (*23*, *24*). These include variable surface antigens that mediate cytoadherence and sequestration of *P. falciparum*–infected red blood cells within capillaries of the brain and placenta, leading to severe disease and death (*22*). Expanded numbers of transporters and extensive horizontal gene transfer in the *Cryptosporidium* genome (*12*–*14*, *25*) may reflect the extreme need for nutrient salvage in this parasite, and provide several attractive targets for this untreatable opportunistic pathogen that afflicts immunosuppressed individuals. Expanded families of secreted *Theileria* proteins may play a role in evading immune recognition or regulating host cell transformation, highlighting targets for drug or vaccine development (*2*). Several individual genes specific to the *Theileria* genome are also suggestive of roles in modulating the host cell cytoskeleton and the immune response (*6*, *7*).

Overall, the range and depth of genomic-scale data sets available for multiple apicomplexan parasite species provide an extraordinarily rich resource for studying the evolution and function of eukaryotic cells, organelles, and host-pathogen interactions.



Parasite life cycles compared. Concentric circles diagram the differentiation of various apicomplexan parasites, as they traverse multiple stages of their complex life cycle. Radial lines indicate distinct invasion events, in which parasites enter new host cells (broken lines correspond to invasion events that do not require entering a new tissue type). Sporozoites enter the mammalian cells indicated in the upper left sector. Theileria transforms lympohocytes to induce a life-threatening lymphoma; other sporozoites produce little pathogenesis, although the hepatocytic stages of some *Plasmodium* species may lie dormant for long periods of time. Merozoites (called tachyzoites in *Toxoplasma*) enter new cells, where they may propagate indefinitely (except for *Cryptosporidium*), causing significant anemia or tissue destruction. *Toxoplasma*-infected cells can also differentiate into latent bradyzoite tissue cysts, particularly in muscle and brain. The pink-shaded region at the bottom indicates sexual stages in ticks (*Theileria*), mosquitoes (*Plasmodium*), or cats (*Toxoplasma*), although *Toxoplasma* bradyzoites may also be transmitted without sexual recombination, via carnivory. *Cryptosporidium* requires no vector species for transmission. The outermost shape depicts a generic merozoite, with rhoptries of the apical complex at the top.

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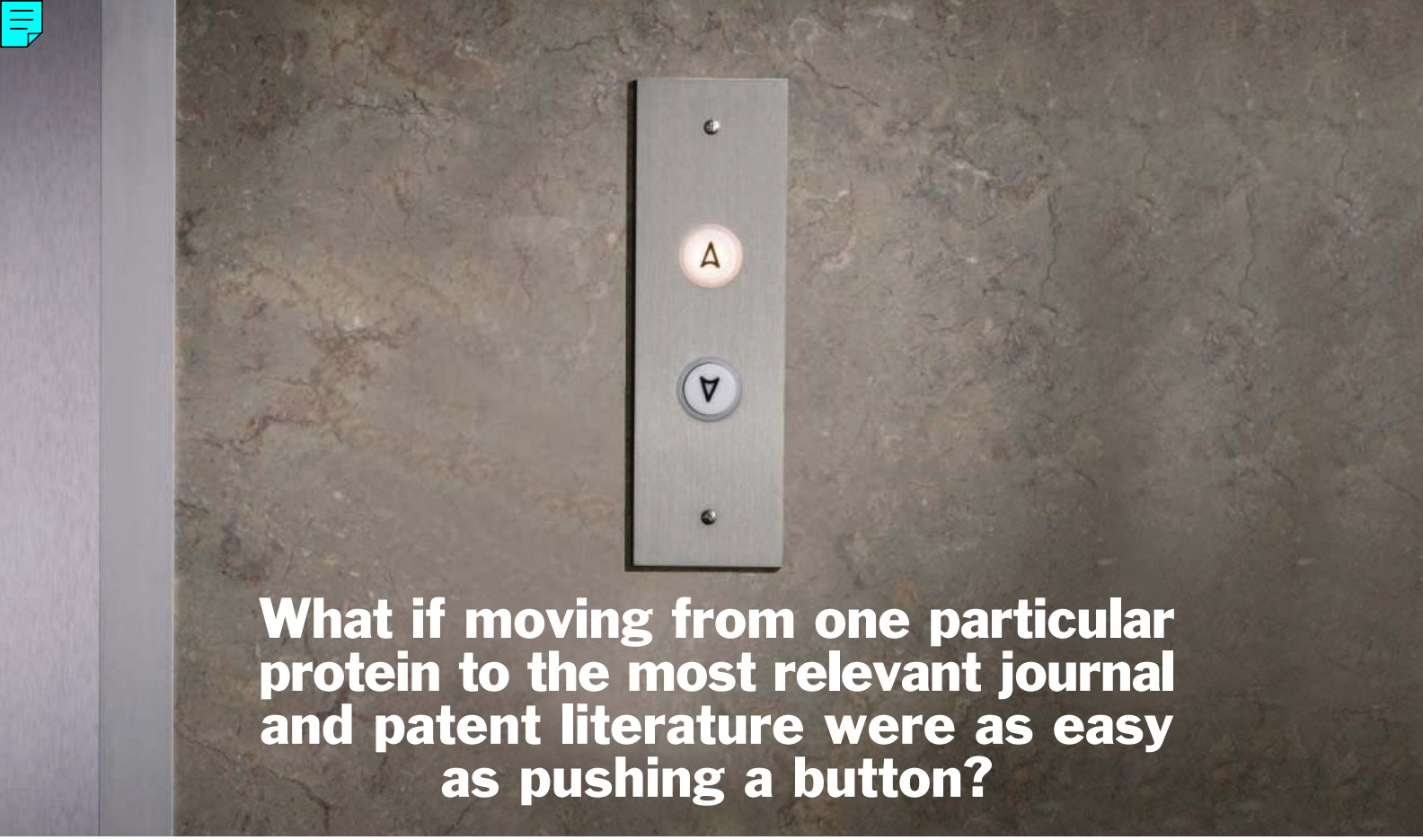

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INTRODUCTION

What Don't We Know?

At *Science*, we tend to get excited about new discoveries that lift the veil a little on how things work, from cells to the universe. That puts our focus firmly on what has been added to our stock of knowledge. For this anniversary issue, we decided to shift our frame of reference, to look instead at what we *don't* know: the scientific puzzles that are driving basic scientific research.

We began by asking *Science*'s Senior Editorial Board, our Board of Reviewing Editors, and our own editors and writers to suggest questions that point to critical knowledge gaps. The ground rules: Scientists should have a good shot at answering the questions over the next 25 years, or they should at least know how to go about answering them. We intended simply to choose 25 of these suggestions and turn them into a survey of the big questions facing science. But when a group of editors and writers sat down to select those big questions, we quickly realized that 25 simply wouldn't convey the grand sweep of cutting-edge research that lies behind the responses we received. So we have ended up with 125 questions, a fitting number for *Science*'s 125th anniversary.

First, a note on what this special issue is not: It is not a survey of the big societal challenges that science can help solve, nor is it a forecast of what science might achieve. Think of it instead as a survey of our scientific ignorance, a broad swath of questions that scientists themselves are asking. As Tom Siegfried puts it in his introductory essay, they are "opportunities to be exploited."

We selected 25 of the 125 questions to highlight based on several criteria: how fundamental they are, how broad-ranging, and whether their solutions will impact other scientific disciplines. Some have few immediate practical implications—the composition of the universe, for example. Others we chose because the answers will have enormous societal impact—whether an effective HIV vaccine is feasible, or how much the carbon dioxide we are pumping into the atmosphere will warm our planet, for example. Some, such as the nature of dark energy, have come to prominence only recently; others, such as the mechanism behind limb regeneration in amphibians, have intrigued scientists for more than a century. We listed the 25 highlighted questions in no special order, but we did group the 100 additional questions roughly by discipline.

Our sister online publications are also devoting special issues to *Science*'s 125th anniversary. The Science of Aging Knowledge Environment, SAGE KE (www.sageke.org), is surveying several big questions confronting researchers on aging. The Signal Transduction Knowledge Environment, STKE (www.stke.org), has selected classic *Science* articles that have had a high impact in the field of cell signaling and is highlighting them in an editorial guide. And *Science*'s Next Wave (www.nextwave.org) is looking at the careers of scientists grappling with some of the questions *Science* has identified.

We are acutely aware that even 125 unknowns encompass only a partial answer to the question that heads this special section: What Don't We Know? So we invite you to participate in a special forum on *Science*'s Web site (www.sciencemag.org/sciext/eletters/125th), in which you can comment on our 125 questions or nominate topics we missed—and we apologize if they are the very questions you are working on.

–DONALD KENNEDY AND COLIN NORMAN

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In Praise of Hard Questions

Great cases, as U.S. Supreme Court Justice Oliver Wendell Holmes suggested a century ago, may make bad law. But great questions often make very good science.

Unsolved mysteries provide science with motivation and direction. Gaps in the road to scientific knowledge are not potholes to be avoided, but opportunities to be exploited.

"Fundamental questions are guideposts; they stimulate people," says 2004 Nobel physics laureate David Gross. "One of the most creative qualities a research scientist can have is the ability to ask the right questions."

Science's greatest advances occur on the frontiers, at the interface between ignorance and knowledge, where the most profound questions are posed. There's no better way to assess the current condition of science than listing the questions that science cannot answer. "Science," Gross declares, "is shaped by ignorance."

There have been times, though, when some believed that science had paved over all the gaps, ending the age of ignorance. When *Science* was born, in 1880, James Clerk Maxwell had died just the year before, after successfully explaining light, electricity, magnetism, and heat. Along with gravity, which Newton had mastered 2 centuries earlier, physics was, to myopic eyes, essentially finished. Darwin, meanwhile, had established the guiding principle of biology, and Mendeleyev's periodic table—only a decade old—allowed chemistry to publish its foundations on a poster board.

Maxwell himself mentioned that many physicists believed the trend in their field was merely to measure the values of physical constants "to another place of decimals."

Nevertheless, great questions raged. Savants of science debated not only the power of natural selection, but also the origin of the solar system, the age and internal structure of Earth, and the prospect of a plurality of worlds populating the cosmos.

In fact, at the time of Maxwell's death, his theory of electromagnetic fields was not yet widely accepted or even well known; experts still argued about whether electricity and magnetism propagated their effects via "action at a distance," as gravity (supposedly) did, or by Michael Faraday's "lines of force" (incorporated by Maxwell into his fields). Lurking behind that dispute was the deeper issue of whether gravity could be unified with electromagnetism (Maxwell thought not), a question that remains one of the greatest in science today, in a somewhat more complicated form.

Maxwell knew full well that his accomplishments left questions unanswered. His calculations regarding the internal motion of molecules did not agree with measurements of specific heats, for instance. "Something essential to the complete state of the physical theory of molecular encounters must have hitherto escaped us," he commented.

When *Science* turned 20—at the 19th century's end—Maxwell's mentor William Thomson (Lord Kelvin) articulated the two grand gaps in knowledge of the day. (He called them "clouds" hanging over physicists' heads.) One was the mystery of specific heats that Maxwell had identified; the other was the failure to detect the ether, a medium seemingly required by Maxwell's electromagnetic waves.

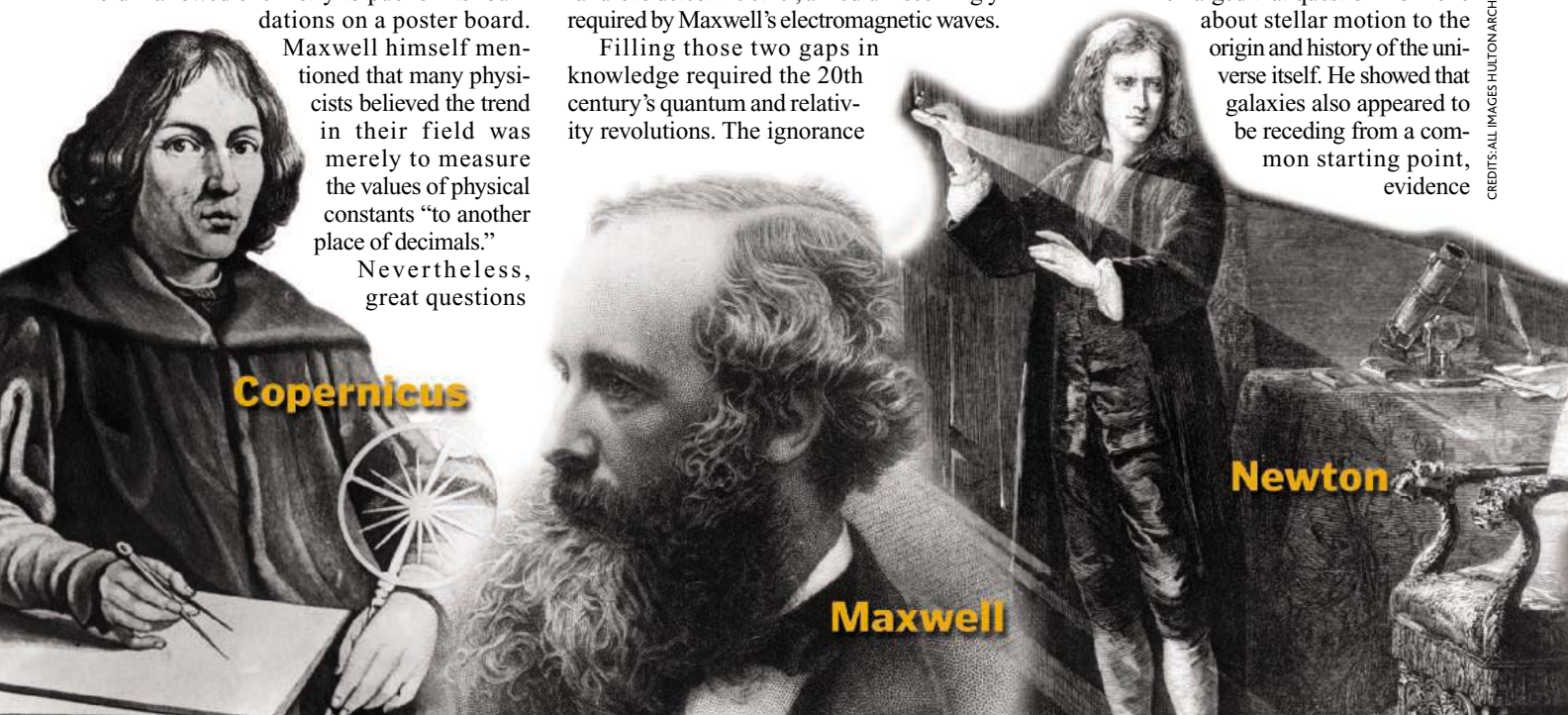
Filling those two gaps in knowledge required the 20th century's quantum and relativity revolutions. The ignorance enveloped in Kelvin's clouds was the impetus for science's revitalization.

Throughout the last century, pursuing answers to great questions reshaped human understanding of the physical and living world. Debates over the plurality of worlds assumed galactic proportions, specifically addressing whether Earth's home galaxy, the Milky Way, was only one of many such conglomerations of stars. That issue was soon resolved in favor of the Milky Way's nonexclusive status, in much the same manner that Earth itself had been demoted from its central role in the cosmos by Copernicus centuries before.

But the existence of galaxies outside our own posed another question, about the apparent motions of those galaxies away from one another. That issue echoed a curious report in *Science*'s first issue about a set of stars forming a triangular pattern, with a double star at the apex and two others forming the base. Precise observations showed the stars to be moving apart, making the triangle bigger but maintaining its form.

"It seems probable that all these stars are slowly moving away from one common point, so that many years back they were all very much closer to one another," *Science* reported, as though the four stars had all begun their journey from the same place. Understanding such motion was a question "of the highest interest."

A half a century later, Edwin Hubble enlarged that question from one about stellar motion to the origin and history of the universe itself. He showed that galaxies also appeared to be receding from a common starting point, evidence



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that the universe was expanding. With Hubble's discovery, cosmology's grand questions began to morph from the philosophical to the empirical. And with the discovery of the cosmic microwave background in the 1960s, the big bang theory of the universe's birth assumed the starring role on the cosmological stage—providing cosmologists with one big answer and many new questions.

By *Science*'s centennial, a quarter-century ago, many gaps still remained in knowledge of the cosmos; some of them have since been filled, while others linger. At that time debate continued over the existence of planets around faraway stars, a question now settled with the discovery of dozens of planets in the solar system's galactic neighborhood. But now a bigger question looms beyond the scope of planets or even galaxies: the prospect of multiple universes, cousins to the bubble of time and space that humans occupy.

And not only may the human universe not be alone (defying the old definition of universe), humans may not be alone in their own space, either. The possible existence of life elsewhere in the cosmos remains as great a gap as any in present-day knowledge. And it is enmeshed with the equally deep mystery of life's origin on Earth.

Life, of course, inspires many deep questions, from the prospects for immortality to the prognosis for eliminating disease. Scientists continue to wonder whether they will ever be able to create new life forms from scratch, or at least simulate life's self-assembling capabilities. Biologists, physicists, mathematicians, and computer scientists have begun cooperating on a sophisticated "systems biology" aimed at understanding how the countless molecular interactions at the heart of life fit together in the workings of cells, organs, and whole animals. And if successful, the systems approach should help doctors tailor treatments to individual variations in DNA, permitting personalized medicine that deters disease without inflicting side effects. Before *Science* turns 150, revamped versions of modern medicine may make it possible for humans to live that long, too.

As *Science* and science age, knowledge and ignorance have coevolved, and the nature of the great questions sometimes changes. Old questions about the age and structure of the Earth, for instance, have given way to issues concerning the planet's capacity to support a growing and aging population.

Some great questions get bigger over time, encompassing an ever-expanding universe, or become more profound, such as the quest to understand consciousness. On the other hand, many deep questions drive science to smaller scales, more minute than the realm of atoms and molecules, or to a greater depth of detail underlying broad-brush answers to past big questions. In 1880, some scientists remained unconvinced by Maxwell's evidence for atoms. Today, the analogous debate focuses on superstrings as the ultimate bits of matter, on a scale a trillion trillion times smaller. Old arguments over evolution and natural selection have descended to debates on the dynamics of speciation, or how particular behaviors, such as altruistic cooperation, have emerged from the laws of individual competition.

Great questions themselves evolve, of course, because their answers spawn new and better questions in turn. The solutions to Kelvin's clouds—relativity and quantum physics—generated many of the mysteries on today's list, from the composition of the cosmos to the prospect for quantum computers.

Ultimately, great questions like these both define the state of scientific knowledge and drive the engines of scientific discovery. Where ignorance and knowledge converge, where the known confronts the unknown, is where scientific progress is most dramatically made. "Thoroughly conscious ignorance," wrote Maxwell, "is the prelude to every real advance in science."

So when science runs out of questions, it would seem, science will come to an end. But there's no real danger of that. The highway from ignorance to knowledge runs both ways: As knowledge accumulates, diminishing the ignorance of the past, new questions arise, expanding the areas of ignorance to explore.

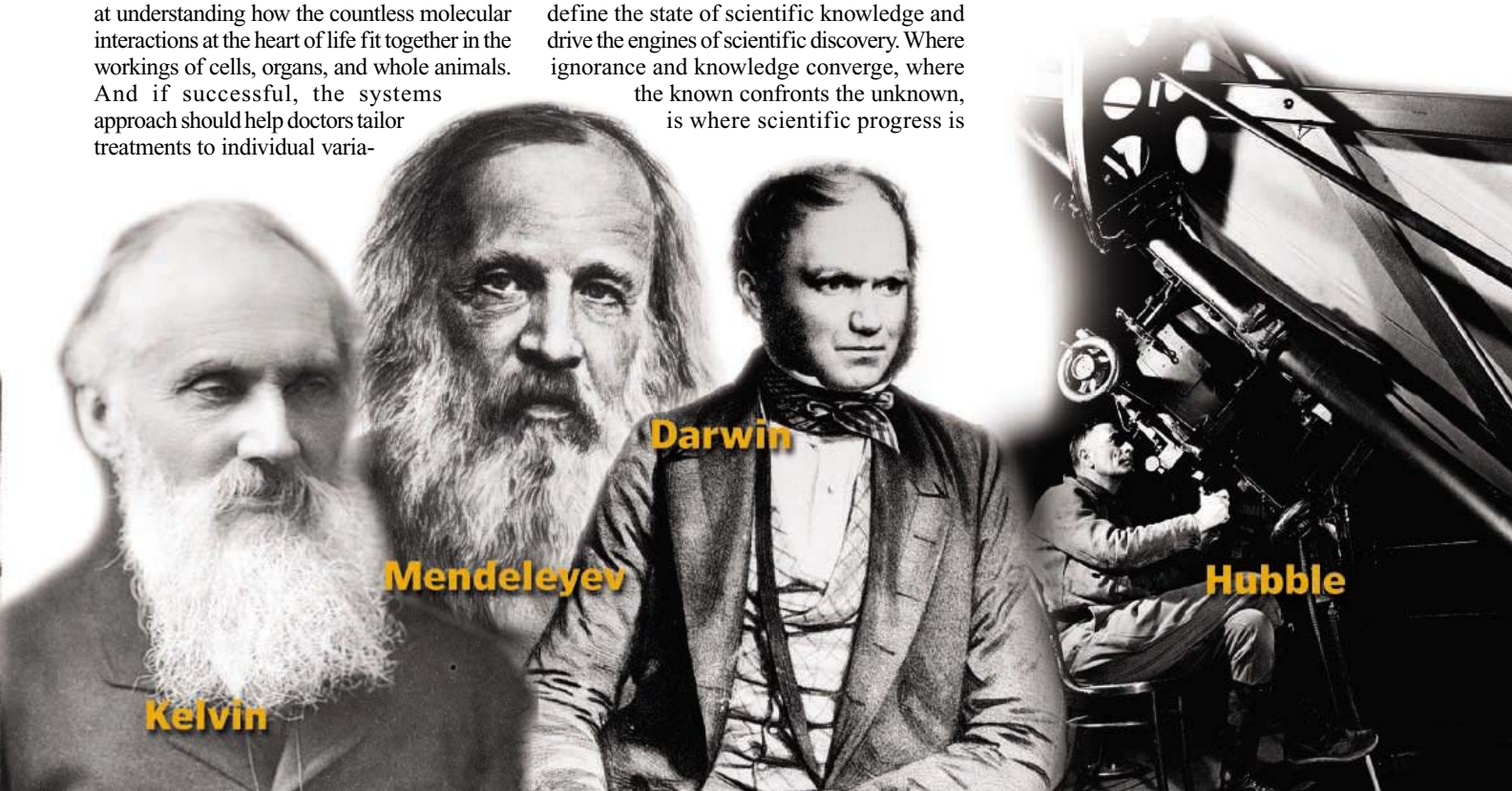
Maxwell knew that even an era of precision measurements is not a sign of science's end but preparation for the opening of new frontiers. In every branch of science, Maxwell declared, "the labor of careful measurement has been rewarded by the discovery of new fields of research and by the development of new scientific ideas."

If science's progress seems to slow, it's because its questions get increasingly difficult, not because there will be no new questions left to answer.

Fortunately, hard questions also can make great science, just as Justice Holmes noted that hard cases, like great cases, made bad law. Bad law resulted, he said, because emotional concerns about celebrated cases exerted pressures that distorted well-established legal principles. And that's why the situation in science is the opposite of that in law. The pressures of the great, hard questions bend and even break well-established principles, which is what makes science forever self-renewing—and which is what demolishes the nonsensical notion that science's job will ever be done.

–TOM SIEGFRIED

Tom Siegfried is the author of *Strange Matters* and *The Bit and the Pendulum*.



What Is the Universe Made Of

Every once in a while, cosmologists are dragged, kicking and screaming, into a universe much more unsettling than they had any reason to expect. In the 1500s and 1600s, Copernicus, Kepler, and Newton showed that Earth is just one of many planets orbiting one of many stars, destroying the comfortable Medieval notion of a closed and tiny cosmos. In the 1920s, Edwin Hubble showed that our universe is constantly expanding and evolving, a finding that eventually shattered the idea that the universe is unchanging and eternal. And in the past few decades, cosmologists have discovered that the ordinary matter that makes up stars and galaxies and people is less than 5% of everything there is. Grappling with this new understanding of the cosmos, scientists face one overriding question: What is the universe made of?

In the dark. Dark matter holds galaxies together; supernovae measurements point to a mysterious dark energy.

This question arises from years of progressively stranger observations. In the 1960s, astronomers discovered that galaxies spun around too fast for the collective pull of the stars' gravity to keep them from flying apart. Something unseen appears to be keeping the stars from flinging themselves away from the center: unilluminated matter that exerts extra gravitational force. This is dark matter.

Over the years, scientists have spotted some of this dark matter in space; they have seen ghostly clouds of gas with x-ray telescopes, watched the twinkle of distant stars as invisible clumps of matter pass in front of them, and measured the distortion of space and time caused by invisible mass in galaxies. And thanks to observations of the abundances of elements in primordial gas clouds, physicists have concluded that only 10% of ordinary matter is visible to telescopes.

But even multiplying all the visible "ordinary" matter by 10 doesn't come close to accounting for how the universe is structured. When astronomers look up in the heavens with powerful telescopes, they see a lumpy cosmos. Galaxies don't dot the skies uniformly; they cluster together in thin tendrils and filaments that twine among vast voids. Just as there isn't enough visible matter to keep galaxies spinning at the right speed, there isn't enough ordinary matter to account for this lumpiness. Cosmologists now conclude that the gravitational forces exerted by another form of dark matter, made of an as-yet-undiscovered type of particle, must be sculpting these vast cosmic structures. They estimate that this exotic dark matter makes up about 25% of the stuff in the universe—five times as much as ordinary matter.

But even this mysterious entity pales by comparison to another mystery: dark energy. In the late 1990s, scientists examining distant supernovae discovered that the universe is expanding faster and faster, instead of slowing down as the laws of physics would imply. Is there some sort of antigravity force blowing the universe up?

All signs point to yes. Independent measurements of a variety of phenomena—cosmic background radiation, element abundances, galaxy clustering, gravitational lensing, gas cloud properties—all converge on a consistent, but bizarre, picture of the cosmos. Ordinary matter and exotic, unknown particles together make up only about 30% of the stuff in the universe; the rest is this mysterious antigravity force known as dark energy.

This means that figuring out what the universe is made of will require answers to three increasingly difficult sets of questions. What is ordinary dark matter made of, and where does it reside? Astrophysical observations, such as those that measure the bending of light by massive objects in space, are already yielding the answer. What is exotic dark matter? Scientists have some ideas, and with luck, a dark-matter trap buried deep underground or a high-energy atom smasher will discover a new type of particle within the next decade. And finally, what is dark energy? This question, which wouldn't even have been asked a decade ago, seems to transcend known physics more than any other phenomenon yet observed. Ever-better measurements of supernovae and cosmic background radiation as well as planned observations of gravitational lensing will yield information about dark energy's "equation of state"—essentially a measure of how squishy the substance is. But at the moment, the nature of dark energy is arguably the murkiest question in physics—and the one that, when answered, may shed the most light.

–CHARLES SEIFE

CREDIT: HIGH-Z SUPERNOVA SEARCH TEAM/HST/NASA

So Much More to Know ... >>

From the nature of the cosmos to the nature of societies, the following 100 questions span the sciences. Some are pieces of questions discussed above; others are big questions in their own right. Some will drive scientific inquiry for the next century; others may soon be answered. Many will undoubtedly spawn new questions.

Is ours the only universe?
A number of quantum theorists and cosmologists are trying to figure out whether our universe is part of a bigger "multiverse." But others suspect that this hard-to-test idea may be a question for philosophers.

What drove cosmic inflation?
In the first moments after the big bang, the universe blew up at an incredible rate. But what did the blowing? Measurements of the cosmic microwave background and other astrophysical observations are narrowing the possibilities.

What Is the Biological Basis of Consciousness

For centuries, debating the nature of consciousness was the exclusive purview of philosophers. But if the recent torrent of books on the topic is any indication, a shift has taken place: Scientists are getting into the game.

Has the nature of consciousness finally shifted from a philosophical question to a scientific one that can be solved by doing experiments? The answer, as with any related to this topic, depends on whom you ask. But scientific interest in this slippery, age-old question seems to be gathering momentum. So far, however, although theories abound, hard data are sparse.

The discourse on consciousness has been hugely influenced by René Descartes, the French philosopher who in the mid–17th century declared that body and mind are made of different stuff entirely. It must be so, Descartes concluded, because the body exists in both time and space, whereas the mind has no spatial dimension.

CREDIT: LESTER LEFKOWITZ/CORBIS

Recent scientifically oriented accounts of consciousness generally reject Descartes's solution; most prefer to treat body and mind as different aspects of the same thing. In this view, consciousness emerges from the properties and organization of neurons in the brain. But how? And how can scientists, with their devotion to objective observation and measurement, gain access to the inherently private and subjective realm of consciousness?

Some insights have come from examining neurological patients whose injuries have altered their consciousness. Damage to certain evolutionarily ancient structures in the brainstem robs people of consciousness entirely, leaving them in a coma or a persistent vegetative state. Although these regions may be a master switch for consciousness, they are unlikely to be its sole source. Different aspects of consciousness are probably generated in different brain regions. Damage to visual areas of the cerebral cortex, for example, can produce strange deficits limited to visual awareness. One extensively studied patient, known as D.F., is unable to identify shapes or determine the orientation of a thin slot in a vertical disk. Yet when asked to pick up a card and slide it through the slot, she does so easily. At some level, D.F. must know the orientation of the slot to be able to do this, but she seems not to know she knows.

Cleverly designed experiments can produce similar dissociations of unconscious and conscious knowledge in people without neurological damage. And researchers hope that scanning the brains of subjects engaged in such tasks will reveal clues about the neural activity required for conscious awareness. Work with monkeys also may elucidate some aspects of consciousness, particularly visual awareness. One experimental approach is to present a monkey with an optical illusion that creates a "bistable percept," looking like one thing one moment and another the next. (The orientation-flipping Necker cube is a well-known example.) Monkeys can be trained to indicate which version they perceive. At the same time, researchers hunt for neurons that track the monkey's perception, in hopes that these neurons will lead them to the neural systems involved in conscious visual awareness and ultimately to an explanation of how a particular pattern of photons hitting the retina produces the experience of seeing, say, a rose.

Experiments under way at present generally address only pieces of the consciousness puzzle, and very few directly address the most enigmatic aspect of the conscious human mind: the sense of self. Yet the experimental work has begun, and if the results don't provide a blinding insight into how consciousness arises from tangles of neurons, they should at least refine the next round of questions.

Ultimately, scientists would like to understand not just the biological basis of consciousness but also why it exists. What selection pressure led to its development, and how many of our fellow creatures share it? Some researchers suspect that consciousness is not unique to humans, but of course much depends on how the term is defined. Biological markers for consciousness might help settle the matter and shed light on how consciousness develops early in life. Such markers could also inform medical decisions about loved ones who are in an unresponsive state.

Until fairly recently, tackling the subject of consciousness was a dubious career move for any scientist without tenure (and perhaps a Nobel Prize already in the bag). Fortunately, more young researchers are now joining the fray. The unanswered questions should keep them—and the printing presses—busy for many years to come.

–GREG MILLER

When and how did the first stars and galaxies form?
The broad brush strokes are visible, but the fine details aren't. Data from satellites and ground-based telescopes may soon help pinpoint, among other particulars, when the first generation of stars burned off the hydrogen "fog" that filled the universe.

JPL/NASA

Where do ultra-high-energy cosmic rays come from?
Above a certain energy, cosmic rays don't travel very far before being destroyed. So why are cosmic-ray hunters spotting such rays with no obvious source within our galaxy?

What powers quasars?
The mightiest energy fountains in the universe probably get their power from matter plunging into whirling supermassive black holes. But the details of what drives their jets remain anybody's guess.

E. J. SCHREIER, STSCI/NASA

What is the nature of black holes?
Relativistic mass crammed into a quantum-sized object? It's a recipe for disaster—and scientists are still trying to figure out the ingredients.

continued >>

WHAT DON'T WE KNOW?

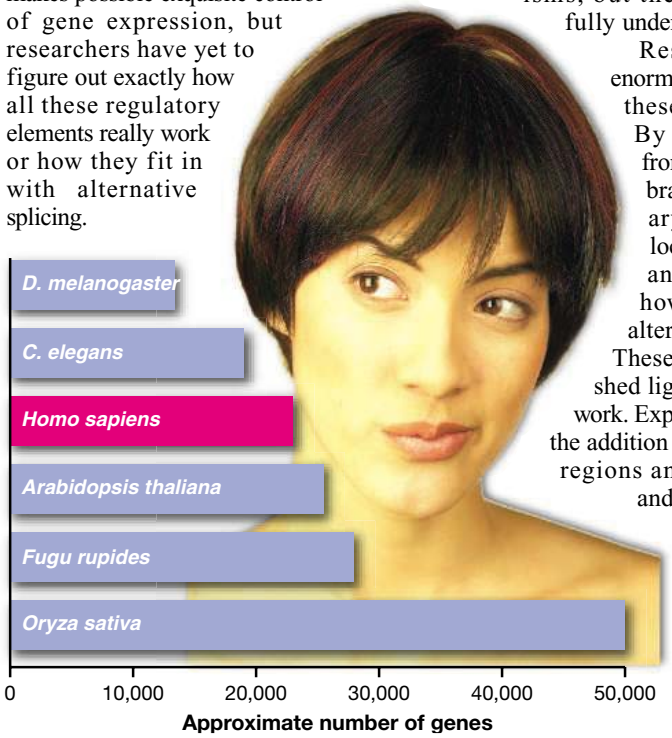
Why Do Humans Have So Few Genes

When leading biologists were unraveling the sequence of the human genome in the late 1990s, they ran a pool on the number of genes contained in the 3 billion base pairs that make up our DNA. Few bets came close. The conventional wisdom a decade or so ago was that we need about 100,000 genes to carry out the myriad cellular processes that keep us functioning. But it turns out that we have only about 25,000 genes—about the same number as a tiny flowering plant called *Arabidopsis* and barely more than the worm *Caenorhabditis elegans*.

That big surprise reinforced a growing realization among geneticists: Our genomes and those of other mammals are far more flexible and complicated than they once seemed. The old notion of one gene/one protein has gone by the board: It is now clear that many genes can make more than one protein. Regulatory proteins, RNA, noncoding bits of DNA, even chemical and structural alterations of the genome itself control how, where, and when genes are expressed. Figuring out how all these elements work together to choreograph gene expression is one of the central challenges facing biologists.

In the past few years, it has become clear that a phenomenon called alternative splicing is one reason human genomes can produce such complexity with so few genes. Human genes contain both coding DNA—exons—and noncoding DNA. In some genes, different combinations of exons can become active at different times, and each combination yields a different protein. Alternative splicing was long considered a rare hiccup during transcription, but researchers have concluded that it may occur in half—some say close to all—of our genes. That finding goes a long way toward explaining how so few genes can produce hundreds of thousands of different proteins. But how the transcription machinery decides which parts of a gene to read at any particular time is still largely a mystery.

The same could be said for the mechanisms that determine which genes or suites of genes are turned on or off at particular times and places. Researchers are discovering that each gene needs a supporting cast of hundreds to get its job done. They include proteins that shut down or activate a gene, for example by adding acetyl or methyl groups to the DNA. Other proteins, called transcription factors, interact with the genes more directly: They bind to landing sites situated near the gene under their control. As with alternative splicing, activation of different combinations of landing sites makes possible exquisite control of gene expression, but researchers have yet to figure out exactly how all these regulatory elements really work or how they fit in with alternative splicing.



In the past decade or so, researchers have also come to appreciate the key roles played by chromatin proteins and RNA in regulating gene expression. Chromatin proteins are essentially the packaging for DNA, holding chromosomes in well-defined spirals. By slightly changing shape, chromatin may expose different genes to the transcription machinery.

Genes also dance to the tune of RNA. Small RNA molecules, many less than 30 bases, now share the limelight with other gene regulators. Many researchers who once focused on messenger RNA and other relatively large RNA molecules have in the past 5 years turned their attention to these smaller cousins, including microRNA and small nuclear RNA. Surprisingly, RNAs in these various guises shut down and otherwise alter gene expression. They also are key to cell differentiation in developing organisms, but the mechanisms are not fully understood.

Researchers have made enormous strides in pinpointing these various mechanisms. By matching up genomes from organisms on different branches on the evolutionary tree, genomicists are locating regulatory regions and gaining insights into how mechanisms such as alternative splicing evolved. These studies, in turn, should shed light on how these regions work. Experiments in mice, such as the addition or deletion of regulatory regions and manipulating RNA, and computer models should also help. But the central question is likely to remain unsolved for a long time: How do all these features meld together to make us whole?

—ELIZABETH PENNISI

CREDIT: JUPITER IMAGES

Why is there more matter than antimatter?
To a particle physicist, matter and antimatter are almost the same. Some subtle difference must explain why matter is common and antimatter rare.

JUPITER IMAGES

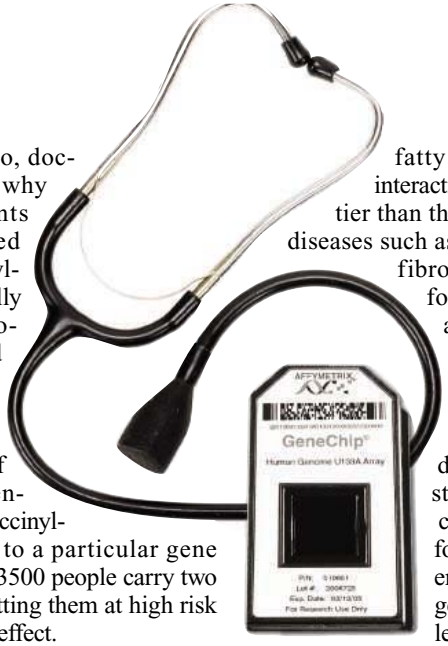
Does the proton decay?
In a theory of everything, quarks (which make up protons) should somehow be convertible to leptons (such as electrons)—so catching a proton decaying into something else might reveal new laws of particle physics.

JUPITER IMAGES

What is the nature of gravity?
It clashes with quantum theory. It doesn't fit in the Standard Model. Nobody has spotted the particle that is responsible for it. Newton's apple contained a whole can of worms.

Why is time different from other dimensions?
It took millennia for scientists to realize that time is a dimension, like the three spatial dimensions, and that time and space are inextricably linked. The equations make sense, but they don't satisfy those who ask why we perceive a "now" or why time seems to flow the way it does.

To What Extent Are Genetic Variation and Personal Health Linked



Forty years ago, doctors learned why some patients who received the anesthetic succinylcholine awoke normally but remained temporarily paralyzed and unable to breathe: They shared an inherited quirk that slowed their metabolism of the drug. Later, scientists traced sluggish succinylcholine metabolism to a particular gene variant. Roughly 1 in 3500 people carry two deleterious copies, putting them at high risk of this distressing side effect.

The solution to the succinylcholine mystery was among the first links drawn between genetic variation and an individual's response to drugs. Since then, a small but growing number of differences in drug metabolism have been linked to genetics, helping explain why some patients benefit from a particular drug, some gain nothing, and others suffer toxic side effects.

The same sort of variation, it is now clear, plays a key role in individual risks of coming down with a variety of diseases. Gene variants have been linked to elevated risks for disorders from Alzheimer's disease to breast cancer, and they may help explain why, for example, some smokers develop lung cancer whereas many others don't.

These developments have led to hopes—and some hype—that we are on the verge of an era of personalized medicine, one in which genetic tests will determine disease risks and guide prevention strategies and therapies. But digging up the DNA responsible—if in fact DNA *is* responsible—and converting that knowledge into gene tests that doctors can use remains a formidable challenge.

Many conditions, including various cancers, heart attacks, lupus, and depression, likely arise when a particular mix of genes collides with something in the environment, such as nicotine or a fatty diet. These multigene interactions are subtler and knottier than the single gene drivers of diseases such as hemophilia and cystic fibrosis; spotting them calls for statistical inspiration and rigorous experiments repeated again and again to guard against introducing unproven gene tests into the clinic. And determining treatment strategies will be no less complex: Last summer, for example, a team of scientists linked 124 different genes to resistance to four leukemia drugs.

But identifying gene networks like these is only the beginning. One of the toughest tasks is replicating these studies—an especially difficult proposition in diseases that are not overwhelmingly heritable, such as asthma, or ones that affect fairly small patient cohorts, such as certain childhood cancers. Many clinical trials do not routinely collect DNA from volunteers, making it sometimes difficult for scientists to correlate disease or drug response with genes. Gene microarrays, which measure expression of dozens of genes at once, can be fickle and supply inconsistent results. Gene studies can also be prohibitively costly.

Nonetheless, genetic dissection of some diseases—such as cancer, asthma, and heart disease—is galloping ahead. Progress in other areas, such as psychiatric disorders, is slower. Severely depressed or schizophrenic patients could benefit enormously from tests that reveal which drug and dose will help them the most, but unlike asthma, drug response can be difficult to quantify biologically, making gene-drug relations tougher to pin down.

As DNA sequence becomes more available and technologies improve, the genetic patterns that govern health will likely come into sharper relief. Genetic tools still under construction, such as a haplotype map that will be used to discern genetic variation behind common diseases, could further accelerate the search for disease genes.

The next step will be designing DNA tests to guide clinical decision-making—and using them. If history is any guide, integrating such tests into standard practice will take time. In emergencies—a heart attack, an acute cancer, or an asthma attack—such tests will be valuable only if they rapidly deliver results.

Ultimately, comprehensive personalized medicine will come only if pharmaceutical companies want it to—and it will take enormous investments in research and development. Many companies worry that testing for genetic differences will narrow their market and squelch their profits.

Still, researchers continue to identify new opportunities. In May, the Icelandic company deCODE Genetics reported that an experimental asthma drug that pharmaceutical giant Bayer had abandoned appeared to decrease the risk of heart attack in more than 170 patients who carried particular gene variants. The drug targets the protein produced by one of those genes. The finding is likely to be just a foretaste of the many surprises in store, as the braids binding DNA, drugs, and disease are slowly unwound.

—JENNIFER COUZIN

CREDITS (TOP TO BOTTOM): JUPITER IMAGES; AFFYMETRIX

Are there smaller building blocks than quarks?
Atoms were "uncuttable." Then scientists discovered protons, neutrons, and other subatomic particles—which were, in turn, shown to be made up of quarks and gluons. Is there something more fundamental still?

CERN

Are neutrinos their own antiparticles?
Nobody knows this basic fact about neutrinos, although a number of underground experiments are under way. Answering this question may be a crucial step to understanding the origin of matter in the universe.

Is there a unified theory explaining all correlated electron systems?
High-temperature superconductors and materials with giant and colossal magnetoresistance are all governed by the collective rather than individual behavior of electrons. There is currently no common framework for understanding them.

continued >>

What is the most powerful laser researchers can build?
Theorists say an intense enough laser field would rip photons into electron-positron pairs, dousing the beam. But no one knows whether it's possible to reach that point.

SANDIA NATIONAL LABORATORY

Can the Laws of Physics Be Unified

At its best, physics eliminates complexity by revealing underlying simplicity. Maxwell's equations, for example, describe all the confusing and diverse phenomena of classical electricity and magnetism by means of four simple rules. These equations are beautiful; they have an eerie symmetry, mirroring one another in an intricate dance of symbols. The four together feel as elegant, as whole, and as complete to a physicist as a Shakespearean sonnet does to a poet.

The Standard Model of particle physics is an unfinished poem. Most of the pieces are there, and even unfinished, it is arguably the most brilliant opus in the literature of physics. With great precision, it describes all known matter—all the subatomic particles such as quarks and leptons—as well as the forces by which those particles interact with one another. These forces are electromagnetism, which describes how charged objects feel each other's influence: the weak force, which explains how particles can change their identities, and the strong force, which describes how quarks stick together to form protons and other composite particles. But as lovely as the Standard Model's description is, it is in pieces, and some of those pieces—those that describe gravity—are missing. It is a few shards of beauty that hint at something greater, like a few lines of Sappho on a fragment of papyrus.

The beauty of the Standard Model is in its symmetry; mathematicians describe its symmetries with objects known as Lie groups. And a mere glimpse at the Standard Model's Lie group betrays its fragmented nature: SU(3) × SU(2) × U(1). Each of those pieces represents one type of symmetry, but the symmetry of the whole is broken. Each of the forces behaves in a slightly different way, so each is described with a slightly different symmetry.

But those differences might be superficial. Electromagnetism and the weak force appear very dissimilar, but in the 1960s physicists showed that at high temperatures, the two forces "unify." It becomes apparent that electromagnetism and the weak force are really the same thing, just as it becomes obvious that ice and liquid water are the same substance if you warm them up together. This connection led physicists to hope that the strong force could also be unified with the other two forces, yielding one large theory described by a single symmetry such as SU(5).

A unified theory should have observable consequences. For example, if the strong force truly is the same as the electroweak force, then protons might not be truly stable; once in a long while, they should decay spontaneously. Despite many searches, nobody has spotted a proton decay, nor has anyone sighted any particles predicted by some symmetry-enhancing modifications to the Standard Model, such as supersymmetry. Worse yet, even such a unified theory can't be complete—as long as it ignores gravity.

Fundamental forces. A theory that ties all four forces together is still lacking.

Gravity is a troublesome force. The theory that describes it, general relativity, assumes that space and time are smooth and continuous, whereas the underlying quantum physics that governs subatomic particles and forces is inherently discontinuous and jumpy. Gravity clashes with quantum theory so badly that nobody has come up with a convincing way to build a single theory that includes all the particles, the strong and electroweak forces, and gravity all in one big bundle. But physicists do have some leads. Perhaps the most promising is superstring theory.

Superstring theory has a large following because it provides a way to unify everything into one large theory with a single symmetry—SO(32) for one branch of superstring theory, for example—but it requires a universe with 10 or 11 dimensions, scads of undetected particles, and a lot of intellectual baggage that might never be verifiable. It may be that there are dozens of unified theories, only one of which is correct, but scientists may never have the means to determine which. Or it may be that the struggle to unify all the forces and particles is a fool's quest.

In the meantime, physicists will continue to look for proton decays, as well as search for supersymmetric particles in underground traps and in the Large Hadron Collider (LHC) in Geneva, Switzerland, when it comes online in 2007. Scientists believe that LHC will also reveal the existence of the Higgs boson, a particle intimately related to fundamental symmetries in the model of particle physics. And physicists hope that one day, they will be able to finish the unfinished poem and frame its fearful symmetry.

—CHARLES SEIFE

CREDITS (TOP TO BOTTOM): JUPITER IMAGES; JUPITER IMAGES; MEHAU KULYK/PHOTO RESEARCHERS INC.; CERN/PHOTO RESEARCHERS INC.

Can researchers make a perfect optical lens?
They've done it with microwaves but never with visible light.

Is it possible to create magnetic semiconductors that work at room temperature?
Such devices have been demonstrated at low temperatures but not yet in a range warm enough for spintronics applications.

What is the pairing mechanism behind high-temperature superconductivity?
Electrons in superconductors surf together in pairs. After 2 decades of intense study, no one knows what holds them together in the complex, high-temperature materials.

Can we develop a general theory of the dynamics of turbulent flows and the motion of granular materials?
So far, such "nonequilibrium systems" defy the tool kit of statistical mechanics, and the failure leaves a gaping hole in physics.

JUPITER IMAGES

JUPITER IMAGES

How Much Can Human Life Span Be Extended

When Jeanne Calment died in a nursing home in southern France in 1997, she was 122 years old, the longest-living human ever documented. But Calment's uncommon status will fade in subsequent decades if the predictions of some biologists and demographers come true. Life-span extension in species from yeast to mice and extrapolation from life expectancy trends in humans have convinced a swath of scientists that humans will routinely coast beyond 100 or 110 years of age. (Today, 1 in 10,000 people in industrialized countries hold centenarian status.) Others say human life span may be far more limited. The elasticity found in other species might not apply to us. Furthermore, testing life-extension treatments in humans may be nearly impossible for practical and ethical reasons.

Just 2 or 3 decades ago, research on aging was a backwater. But when molecular biologists began hunting for ways to prolong life, they found that life span was remarkably pliable. Reducing the activity of an insulinlike receptor more than doubles the life span of worms to a startling—for them—6 weeks. Put certain strains of mice on near-starvation but nutrient-rich diets, and they live 50% longer than normal.

Some of these effects may not occur in other species. A worm's ability to enter a "dauer" state, which resembles hibernation, may be critical, for example. And shorter-lived species such as worms and fruit flies, whose aging has been delayed the most, may be more susceptible to life-span manipulation. But successful approaches are converging on a few key areas: calorie restriction; reducing levels of insulinlike growth factor 1 (IGF-1), a protein; and preventing oxidative damage to the body's tissues. All three might be interconnected, but so far that hasn't been confirmed (although calorie-restricted animals have low levels of IGF-1).

Can these strategies help humans live longer? And how do we determine whether they will? Unlike drugs for cancer or heart disease, the benefits of antiaging treatments are fuzzier, making studies difficult to set up and to interpret. Safety is uncertain; calorie restriction reduces fertility in animals, and lab flies bred to live long can't compete with their wild counterparts. Furthermore, garnering results—particularly from younger volunteers, who may be likeliest to benefit because they've aged the least—will take so long that by the time results are in, those who began the study will be dead.

That hasn't stopped scientists, some of whom have founded companies, from searching for treatments to slow aging. One intriguing question is whether calorie restriction works in humans. It's being tested in primates, and the National Institute on Aging in Bethesda, Maryland, is funding short-term studies in people. Volunteers in those trials have been on a stringent diet for up to 1 year while researchers monitor their metabolism and other factors that could hint at how they're aging.

Insights could also come from genetic studies of centenarians, who may have inherited long life from their parents. Many scientists believe that average human life span has an inherent upper limit, although they don't agree on whether it's 85 or 100 or 150.

One abiding question in the antiaging world is what the goal of all this work ought to be. Overwhelmingly, scientists favor treatments that will slow aging and stave off age-related diseases rather than simply extending life at its most decrepit. But even so, slowing aging could have profound social effects, upsetting actuarial tables and retirement plans.

Then there's the issue of fairness: If antiaging therapies become available, who will receive them? How much will they cost? Individuals may find they can stretch their life spans. But that may be tougher to achieve for whole populations, although many demographers believe that the average life span will continue to climb as it has consistently for decades. If that happens, much of the increase may come from less dramatic strategies, such as heart disease and cancer prevention, that could also make the end of a long life more bearable. —JENNIFER COUZIN

CREDIT: GETTY IMAGES

Are there stable high-atomic-number elements?
A superheavy element with 184 neutrons and 114 protons should be relatively stable, if physicists can create it.

Is superfluidity possible in a solid? If so, how?
Despite hints in solid helium, nobody is sure whether a crystalline material can flow without resistance. If new types of experiments show that such outlandish behavior is possible, theorists would have to explain how.

What is the structure of water?
Researchers continue to tussle over how many bonds each H_2O molecule makes with its nearest neighbors.

JUPITER IMAGES

What is the nature of the glassy state?
Molecules in a glass are arranged much like those in liquids but are more tightly packed. Where and why does liquid end and glass begin?

continued >>

Are there limits to rational chemical synthesis?
The larger synthetic molecules get, the harder it is to control their shapes and make enough copies of them to be useful. Chemists will need new tools to keep their creations growing.

What Controls Organ Regeneration

Unlike automobiles, humans get along pretty well for most of their lives with their original parts. But organs do sometimes fail, and we can't go to the mechanic for an engine rebuild or a new water pump—at least not yet. Medicine has battled back many of the acute threats, such as infection, that curtailed human life in past centuries. Now, chronic illnesses and deteriorating organs pose the biggest drain on human health in industrialized nations, and they will only increase in importance as the population ages. Regenerative medicine—rebuilding organs and tissues—could conceivably be the 21st century equivalent of antibiotics in the 20th. Before that can happen, researchers must understand the signals that control regeneration.

Researchers have puzzled for centuries over how body parts replenish themselves. In the mid-1700s, for instance, Swiss researcher Abraham Trembley noted that when chopped into pieces, hydra—tubelike creatures with tentacles that live in fresh water—could grow back into complete, new organisms. Other scientists of the era examined the salamander's ability to replace a severed tail. And a century later, Thomas Hunt Morgan scrutinized planaria, flatworms that can regenerate even when whittled into 279 bits. But he decided that regeneration was an intractable problem and forsook planaria in favor of fruit flies.

Mainstream biology has followed in Morgan's wake, focusing on animals suitable for studying genetic and embryonic development. But some researchers have pressed on with studies of regeneration superstars, and they've devised innovative strategies to tackle the genetics of these organisms. These efforts and investigations of new regeneration models—such as zebrafish and special mouse lines—are beginning to reveal the forces that guide regeneration and those that prevent it.

Animals exploit three principal strategies to regenerate organs. First, working organ cells that normally don't divide can multiply and grow to replenish lost tissue, as occurs in injured salamander hearts. Second, specialized cells can undo their training—a process known as dedifferentiation—and assume a more pliable form that can replicate and later respecialize to reconstruct a missing part. Salamanders and newts take this approach to heal and rebuild a severed limb, as do zebrafish to mend clipped fins. Finally, pools of stem cells can step in to perform required renovations. Planaria tap into this resource when reconstructing themselves.

Self-repair. Newts reprogram their cells to reconstruct a severed limb.

Humans already plug into these mechanisms to some degree. For instance, after surgical removal of part of a liver, healing signals tell remaining liver cells to resume growth and division to expand the organ back to its original size. Researchers have found that when properly enticed, some types of specialized human cells can revert to a more nascent state (see p. 85). And stem cells help replenish our blood, skin, and bones. So why do our hearts fill with scar tissue, our lenses cloud, and our brain cells perish?

Animals such as salamanders and planaria regenerate tissues by rekindling genetic mechanisms that guide the patterning of body structures during embryonic development. We employ similar pathways to shape our parts as embryos, but over the course of evolution, humans may have lost the ability to tap into it as adults, perhaps because the cell division required for regeneration elevated the likelihood of cancer. And we may have evolved the capacity to heal wounds rapidly to repel infection, even though speeding the pace means more scarring. Regeneration pros such as salamanders heal wounds methodically and produce pristine tissue. Avoiding fibrotic tissue could mean the difference between regenerating and not: Mouse nerves grow vigorously if experimentally severed in a way that prevents scarring, but if a scar forms, nerves wither.

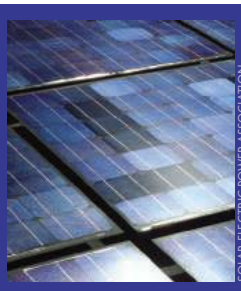
Unraveling the mysteries of regeneration will depend on understanding what separates our wound-healing process from that of animals that are able to regenerate. The difference might be subtle: Researchers have identified one strain of mice that seals up ear holes in weeks, whereas typical strains never do. A relatively modest number of genetic differences seems to underlie the effect. Perhaps altering a handful of genes would be enough to turn us into superhealers, too. But if scientists succeed in initiating the process in humans, new questions will emerge. What keeps regenerating cells from running amok? And what ensures that regenerated parts are the right size and shape, and in the right place and orientation? If researchers can solve these riddles—and it's a big "if"—people might be able to order up replacement parts for themselves, not just their '67 Mustangs.

–R. JOHN DAVENPORT

R. John Davenport is an editor of *Science*'s SAGE KE.

CREDIT: J. P. BROCKES AND A. KUMAR, *NATURE REVIEWS MOLECULAR CELL BIOLOGY* 3, 566 (2002)

What is the ultimate efficiency of photovoltaic cells?
Conventional solar cells top out at converting 32% of the energy in sunlight to electricity. Can researchers break through the barrier?



SOLAR ELECTRIC POWER ASSOCIATION

Will fusion always be the energy source of the future?
It's been 35 years away for about 50 years, and unless the international community gets its act together, it'll be 35 years away for many decades to come.

What drives the solar magnetic cycle?
Scientists believe differing rates of rotation from place to place on the sun underlie its 22-year sunspot cycle. They just can't make it work in their simulations. Either a detail is askew, or it's back to the drawing board.

How do planets form?
How bits of dust and ice and gobs of gas came together to form the planets without the sun devouring them all is still unclear. Planetary systems around other stars should provide clues.

JPL/NASA

How Can a Skin Cell Become a Nerve Cell

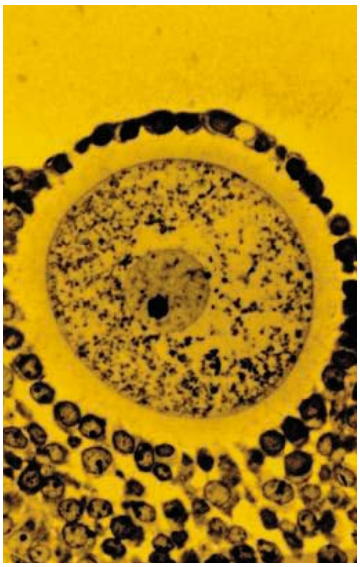
Like Medieval alchemists who searched for an elixir that could turn base metals into gold, biology's modern alchemists have learned how to use oocytes to turn normal skin cells into valuable stem cells, and even whole animals. Scientists, with practice, have now been able to make nuclear transfer nearly routine to produce cattle, cats, mice, sheep, goats, pigs, and—as a Korean team announced in May—even human embryonic stem (ES) cells. They hope to go still further and turn the stem cells into treatments for previously untreatable diseases. But like the medieval alchemists, today's cloning and stem cell biologists are working largely with processes they don't fully understand: What actually happens inside the oocyte to reprogram the nucleus is still a mystery, and scientists have a lot to learn before they can direct a cell's differentiation as smoothly as nature's program of development does every time a fertilized egg gives rise to the multiple cell types that make up a live baby.

Scientists have been investigating the reprogramming powers of the oocyte for half a century. In 1957, developmental biologists first discovered that they could insert the nucleus of adult frog cells into frog eggs and create dozens of genetically identical tadpoles. But in 50 years, the oocyte has yet to give up its secrets.

The answers lie deep in cell biology. Somehow, scientists know, the genes that control development—generally turned off in adult cells—get turned back on again by the oocyte, enabling the cell to take on the youthful potential of a newly fertilized egg. Scientists understand relatively little about these on-and-off switches in normal cells, however, let alone the unusual reversal that takes place during nuclear transfer.

As cells differentiate, their DNA becomes more tightly packed, and genes that are no longer needed—or those which should not be expressed—are blocked. The DNA wraps tightly around proteins called histones, and genes are then tagged with methyl groups that prevent the proteinmaking machinery in the cell from reaching them. Several studies have shown that enzymes that remove those methyl groups are crucial for nuclear transfer to work. But they are far from the only things that are needed.

If scientists could uncover the oocyte's secrets, it might be possible to replicate its tricks without using oocytes themselves, a resource that is fairly difficult to obtain and the use of which raises numerous ethical questions. If scientists could come up with a cell-free bath that turned the clock back on already-differentiated cells, the implications could be enormous. Labs could rejuvenate cells from patients and perhaps then grow them into new tissue that could repair parts worn out by old age or disease.



Cellular alchemist. A human oocyte.

CREDIT: DON W. FAWCETT/VISUALS UNLIMITED

But scientists are far from sure if such cell-free alchemy is possible. The egg's very structure, its scaffolding of proteins that guide the chromosomes during cell division, may also play a key role in turning on the necessary genes. If so, developing an elixir of proteins that can turn back a cell's clock may remain elusive.

To really make use of the oocyte's power, scientists still need to learn how to direct the development of the rejuvenated stem cells and guide them into forming specific tissues. Stem cells, especially those from embryos, spontaneously form dozens of cell types, but controlling that development to produce a single type of cell has proved more difficult. Although some teams have managed to produce nearly pure colonies of certain kinds of neural cells from ES cells, no one has managed to concoct a recipe that will direct the cells to become, say, a pure population of dopamine-producing neurons that could replace those missing in Parkinson's disease.

Scientists are just beginning to understand how cues interact to guide a cell toward its final destiny. Decades of work in developmental biology have provided a start: Biologists have used mutant frogs, flies, mice, chicks, and fish to identify some of the main genes that control a developing cell's decision to become a bone cell or a muscle cell. But observing what goes wrong when a gene is missing is easier than learning to orchestrate differentiation in a culture dish. Understanding how the roughly 25,000 human genes work together to form tissues—and tweaking the right ones to guide an immature cell's development—will keep researchers occupied for decades. If they succeed, however, the result will be worth far more than its weight in gold.

–GRETCHEN VOGEL

What causes ice ages?
Something about the way the planet tilts, wobbles, and careens around the sun presumably brings on ice ages every 100,000 years or so, but reams of climate records haven't explained exactly how.

What causes reversals in Earth's magnetic field?
Computer models and laboratory experiments are generating new data on how Earth's magnetic poles might flip-flop. The trick will be matching simulations to enough aspects of the magnetic field beyond the inaccessible core to build a convincing case.

Are there earthquake precursors that can lead to useful predictions?
Prospects for finding signs of an imminent quake have been waning since the 1970s. Understanding faults will progress, but routine prediction would require an as-yet-unimagined breakthrough.

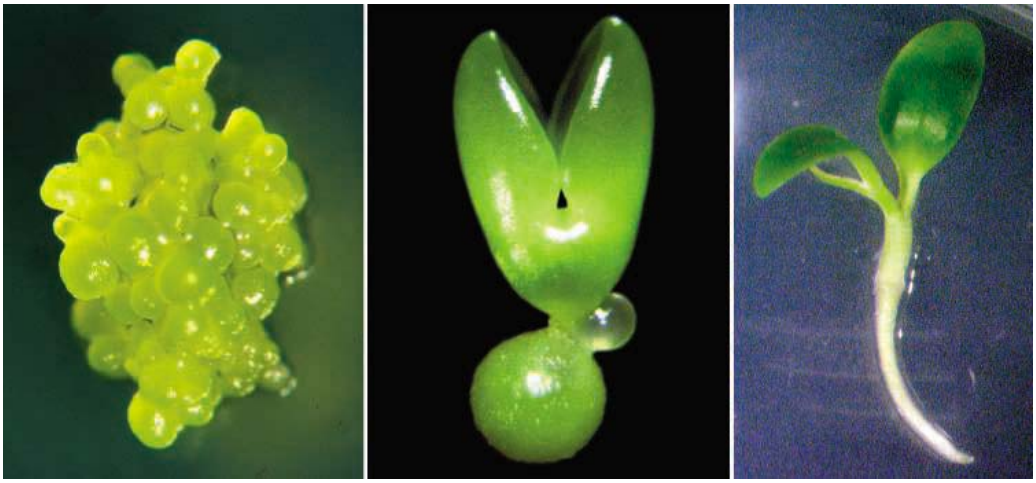
USGS

Is there—or was there—life elsewhere in the solar system?
The search for life—past or present—on other planetary bodies now drives NASA's planetary exploration program, which focuses on Mars, where water abounded when life might have first arisen.

continued >>

How Does a Single Somatic Cell Become A Whole Plant

It takes a certain amount of flexibility for a plant to survive and reproduce. It can stretch its roots toward water and its leaves toward sunlight, but it has few options for escaping predators or finding mates. To compensate, many plants have evolved repair mechanisms and reproductive strategies that allow them to produce offspring even without the meeting of sperm and egg. Some can reproduce from outgrowths of stems, roots, and bulbs, but others are even more radical, able to create new embryos from single somatic cells. Most citrus trees, for example, can form embryos from the tissues surrounding the unfertilized gametes—a feat no animal can manage. The houseplant *Bryophyllum* can sprout embryos from the edges of its leaves, a bit like Athena springing from Zeus's head.



Power of one. Orange tree embryos can sprout from a single somatic cell.

Nearly 50 years ago, scientists learned that they could coax carrot cells to undergo such embryogenesis in the lab. Since then, people have used so-called somatic embryogenesis to propagate dozens of species, including coffee, magnolias, mangos, and roses. A Canadian company has planted entire forests of fir trees that started life in tissue culture. But like researchers who clone animals (see p. 85), plant scientists understand little about what actually controls the process. The search for answers might shed light on how cells' fates become fixed during development, and how plants manage to retain such flexibility.

Scientists aren't even sure which cells are capable of embryogenesis. Although earlier work assumed that all plant cells were equally labile, recent evidence suggests that only a subset of cells can transform into embryos. But what those cells look like before their transformation is a mystery. Researchers have videotaped cultures in which embryos develop but found no visual pattern that hints at which cells are about to sprout, and staining for certain patterns of gene expression has been inconclusive.

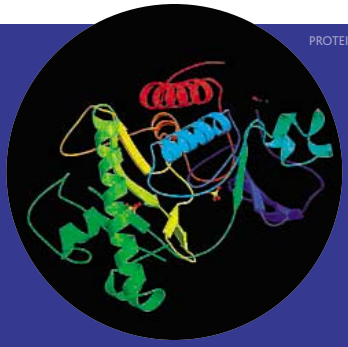
Researchers do have a few clues about the molecules that might be involved. In the lab, the herbicide 2,4-dichlorophenoxyacetic acid (sold as weed killer and called 2,4-D) can prompt cells in culture to elongate, build a new cell wall, and start dividing to form embryos. The herbicide is a synthetic analog of the plant hormones called auxins, which control everything from the plant's response to light and gravity to the ripening of fruit. Auxins might also be important in natural somatic embryogenesis: Embryos that sprout on top of veins near the leaf edge are exposed to relatively high levels of auxins. Recent work has also shown that over- or underexpression of certain genes in *Arabidopsis* plants can prompt embryogenesis in otherwise normal-looking leaf cells.

Sorting out sex-free embryogenesis might help scientists understand the cellular switches that plants use to stay flexible while still keeping growth under control. Developmental biologists are keen to learn how those mechanisms compare in plants and animals. Indeed, some of the processes that control somatic embryogenesis may be similar to those that occur during animal cloning or limb regeneration (see p. 84).

On a practical level, scientists would like to be able to use lab-propagation techniques on crop plants such as maize that still require normal pollination. That would speed up both breeding of new varieties and the production of hybrid seedlings—a flexibility that farmers and consumers could both appreciate. –GRETCHEN VOGEL

CREDITS: USHRL/ARS/USDA

What is the origin of homochirality in nature?
Most biomolecules can be synthesized in mirror-image shapes. Yet in organisms, amino acids are always left-handed, and sugars are always right-handed. The origins of this preference remain a mystery.



PROTEIN DATA BANK

Can we predict how proteins will fold?
Out of a near infinitude of possible ways to fold, a protein picks one in just tens of microseconds. The same task takes 30 years of computer time.

How many proteins are there in humans?
It has been hard enough counting genes. Proteins can be spliced in different ways and decorated with numerous functional groups, all of which makes counting their numbers impossible for now.

How do proteins find their partners?
Protein-protein interactions are at the heart of life. To understand how partners come together in precise orientations in seconds, researchers need to know more about the cell's biochemistry and structural organization.

A long way to go. Grasping the workings of plate tectonics will require deeper probing.

CREDIT: ROGER RESSMEYER/CORBIS

How Does Earth's Interior Work

The plate tectonics revolution went only so deep. True, it made wonderful sense of most of the planet's geology. But that's something like understanding the face of Big Ben; there must be a lot more inside to understand about how and why it all works. In the case of Earth, there's another 6300 kilometers of rock and iron beneath the tectonic plates whose churnings constitute the inner workings of a planetary heat engine. Tectonic plates jostling about the surface are like the hands sweeping across the clock face: informative in many ways but largely mute as to what drives them.

Earth scientists inherited a rather simple picture of Earth's interior from their pre–plate tectonics colleagues. Earth was like an onion. Seismic waves passing through the deep Earth suggested that beneath the broken skin of plates lies a 2800-kilometer layer of rocky mantle overlying 3470 kilometers of molten and—at the center—solid iron. The mantle was further subdivided at a depth of 670 kilometers into upper and lower layers, with a hint of a layer a couple of hundred kilometers thick at the bottom of the lower mantle.

In the postrevolution era, the onion model continued to loom large. The dominant picture of Earth's inner workings divided the planet at the 670-kilometer depth, forming with the core a three-layer machine. Above the 670, the mantle churned slowly like a very shallow pot of boiling water, delivering heat and rock at mid-ocean ridges to make new crust and cool the interior and accepting cold sinking slabs of old plate at deep-sea trenches. A plume of hot rock might rise from just above the 670 to form a volcanic hot spot like Hawaii. But no hot rock rose up through the 670 barrier, and no cold rock sank down through it. Alternatively, argued a smaller contingent, the mantle churned from bottom to top like a deep stockpot, with plumes rising all the way from the core-mantle boundary.

Forty years of probing inner Earth with ever more sophisticated seismic imaging has boosted the view of the engine's complexity without much calming the debate about how it works. Imaging now clearly shows that the 670 is no absolute barrier. Slabs penetrate the boundary, although with difficulty. Layered-earth advocates have duly dropped their impenetrable boundary to 1000 kilometers or deeper. Or maybe there's a flexible, semipermeable boundary somewhere that limits mixing to only the most insistent slabs or plumes.

Now seismic imaging is also outlining two great globs of mantle rock standing beneath Africa and the Pacific like pistons. Researchers disagree whether they are hotter than average and rising under their own buoyancy, denser and sinking, or merely passively being carried upward by adjacent currents. Thin lenses of partially melted rock dot the mantle bottom, perhaps marking the bottom of plumes, or perhaps not. Geochemists reading the entrails of elements and isotopes in mantle-derived rocks find signs of five long-lived "reservoirs" that must have resisted mixing in the mantle for billions of years. But they haven't a clue where in the depths of the mantle those reservoirs might be hiding.

How can we disassemble the increasingly complex planetary machine and find what makes it tick? With more of the same, plus a large dose of patience. After all, plate tectonics was more than a half-century in the making, and those revolutionaries had to look little deeper than the sea floor.

Seismic imaging will continue to improve as better seismometers are spread more evenly about the globe. Seismic data are already distinguishing between temperature and compositional effects, painting an even more complex picture of mantle structure. Mineral physicists working in the lab will tease out more properties of rock under deep mantle conditions to inform interpretation of the seismic data, although still handicapped by the uncertain details of mantle composition. And modelers will more faithfully simulate the whole machine, drawing on seismics, mineral physics, and subtle geophysical observations such as gravity variations. Another 40 years should do it.

–RICHARD A. KERR

How many forms of cell death are there?
In the 1970s, apoptosis was finally recognized as distinct from necrosis. Some biologists now argue that the cell death story is even more complicated. Identifying new ways cells die could lead to better treatments for cancer and degenerative diseases.

What keeps intracellular traffic running smoothly?
Membranes inside cells transport key nutrients around, and through, various cell compartments without sticking to each other or losing their way. Insights into how membranes stay on track could help conquer diseases, such as cystic fibrosis.

KATHARINE SUTLIFF/*SCIENCE*

What enables cellular components to copy themselves independent of DNA?
Centrosomes, which help pull apart paired chromosomes, and other organelles replicate on their own time, without DNA's guidance. This independence still defies explanation.

C. SLAYDEN/*SCIENCE*

continued >>

What roles do different forms of RNA play in genome function?
RNA is turning out to play a dizzying assortment of roles, from potentially passing genetic information to offspring to muting gene expression. Scientists are scrambling to decipher this versatile molecule.

Are We Alone In the Universe

Alone, in all that space? Not likely. Just do the numbers: Several hundred billion stars in our galaxy, hundreds of billions of galaxies in the observable universe, and 150 planets spied already in the immediate neighborhood of the sun. That should make for plenty of warm, scummy little ponds where life could come together to begin billions of years of evolution toward technology-wielding creatures like ourselves. No, the really big question is when, if ever, we'll have the technological wherewithal to reach out and touch such intelligence. With a bit of luck, it could be in the next 25 years.

Workers in the search for extraterrestrial intelligence (SETI) would have needed more than a little luck in the first 45 years of the modern hunt for like-minded colleagues out there. Radio astronomer Frank Drake's landmark Project Ozma was certainly a triumph of hope over daunting odds. In 1960, Drake pointed a 26-meter radio telescope dish in Green Bank, West Virginia, at two stars for a few days each. Given the vacuum-tube technology of the time, he could scan across 0.4 megahertz of the microwave spectrum one channel at a time.

Almost 45 years later, the SETI Institute in Mountain View, California, completed its 10-year-long Project Phoenix. Often using the 350-meter antenna at Arecibo, Puerto Rico, Phoenix researchers searched 710 star systems at 28 million channels simultaneously across an 1800-megahertz range. All in all, the Phoenix search was 100 trillion times more effective than Ozma was.

Besides stunning advances in search power, the first 45 years of modern SETI have also seen a diversification of search strategies. The Search for Extraterrestrial Radio Emissions from Nearby Developed Intelligent Populations (SERENDIP) has scanned billions of radio sources in the Milky Way by piggybacking receivers on antennas in use by observational astronomers, including Arecibo. And other groups are turning modest-sized optical telescopes to searching for nanosecond flashes from alien lasers.

Listening for E.T. The SETI Institute is deploying an array of antennas and tying them into a giant "virtual telescope."

Still, nothing has been heard. But then, Phoenix, for example, scanned just one or two nearby sunlike stars out of each 100 million stars out there. For such sparse sampling to work, advanced, broadcasting civilizations would have to be abundant, or searchers would have to get very lucky.

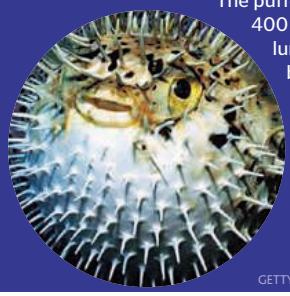
To find the needle in a galaxy-size haystack, SETI workers are counting on the consistently exponential growth of computing power to continue for another couple of decades. In northern California, the SETI Institute has already begun constructing an array composed of individual 6-meter antennas. Ever-cheaper computer power will eventually tie 350 such antennas into "virtual telescopes," allowing scientists to search many targets at once. If Moore's law—that the cost of computation halves every 18 months—holds for another 15 years or so, SETI workers plan to use this antenna array approach to check out not a few thousand but perhaps a few million or even tens of millions of stars for alien signals. If there were just 10,000 advanced civilizations in the galaxy, they could well strike pay dirt before *Science* turns 150.

The technology may well be available in coming decades, but SETI will also need money. That's no easy task in a field with as high a "giggle factor" as SETI has. The U.S. Congress forced NASA to wash its hands of SETI in 1993 after some congressmen mocked the whole idea of spending federal money to look for "little green men with misshapen heads," as one of them put it. Searching for another tippy-top branch of the evolutionary tree still isn't part of the NASA vision. For more than a decade, private funding alone has driven SETI. But the SETI Institute's planned $35 million array is only a prototype of the Square Kilometer Array that would put those tens of millions of stars within reach of SETI workers. For that, mainstream radio astronomers will have to be onboard—or we'll be feeling alone in the universe a long time indeed.

–RICHARD A. KERR

CREDIT: SETI

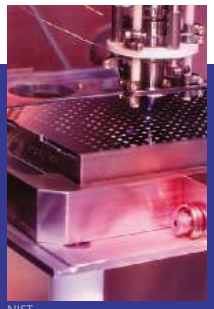
What role do telomeres and centromeres play in genome function?
These chromosome features will remain mysteries until new technologies can sequence them.



GETTY IMAGES

Why are some genomes really big and others quite compact?
The puffer fish genome is 400 million bases; one lungfish's is 133 billion bases long. Repetitive and duplicated DNA don't explain why this and other size differences exist.

What is all that "junk" doing in our genomes?
DNA between genes is proving important for genome function and the evolution of new species. Comparative sequencing, microarray studies, and lab work are helping genomicists find a multitude of genetic gems amid the junk.



NIST

How much will new technologies lower the cost of sequencing?
New tools and conceptual breakthroughs are driving the cost of DNA sequencing down by orders of magnitude. The reductions are enabling research from personalized medicine to evolutionary biology to thrive.

How and Where Did Life on Earth Arise

For the past 50 years, scientists have attacked the question of how life began in a pincer movement. Some approach it from the present, moving backward in time from life today to its simpler ancestors. Others march forward from the formation of Earth 4.55 billion years ago, exploring how lifeless chemicals might have become organized into living matter.

Working backward, paleontologists have found fossils of microbes dating back at least 3.4 billion years. Chemical analysis of even older rocks suggests that photosynthetic organisms were already well established on Earth by 3.7 billion years ago. Researchers suspect that the organisms that left these traces shared the same basic traits found in all life today. All free-living organisms encode genetic information in DNA and catalyze chemical reactions using proteins. Because DNA and proteins depend so intimately on each other for their survival, it's hard to imagine one of them having evolved first. But it's just as implausible for them to have emerged simultaneously out of a prebiotic soup.

Experiments now suggest that earlier forms of life could have been based on a third kind of molecule found in today's organisms: RNA. Once considered nothing more than a cellular courier, RNA turns out to be astonishingly versatile, not only encoding genetic information but also acting like a protein. Some RNA molecules switch genes on and off, for example, whereas others bind to proteins and other molecules. Laboratory experiments suggest that RNA could have replicated itself and carried out the other functions required to keep a primitive cell alive.

Only after life passed through this "RNA world," many scientists now agree, did it take on a more familiar cast. Proteins are thousands of times more efficient as a catalyst than RNA is, and so once they emerged they would have been favored by natural selection. Likewise, genetic information can be replicated from DNA with far fewer errors than it can from RNA.

Other scientists have focused their efforts on figuring out how the lifeless chemistry of a prebiotic Earth could have given rise to an RNA world. In 1953, working at the University of Chicago, Stanley Miller and Harold Urey demonstrated that experiments could shed light on this question. They ran an electric current through a mix of ammonia, methane, and other gases believed at the time to have been present on early Earth. They found that they could produce amino acids and other important building blocks of life.

Cauldron of life? Deep-sea vents are one proposed site for life's start.

CREDIT: DAVID A. HARDY/PHOTO RESEARCHERS INC.

Today, many scientists argue that the early atmosphere was dominated by other gases, such as carbon dioxide. But experiments in recent years have shown that under these conditions, many building blocks of life can be formed. In addition, comets and meteorites may have delivered organic compounds from space.

Just where on Earth these building blocks came together as primitive life forms is a subject of debate. Starting in the 1980s, many scientists argued that life got its start in the scalding, mineral-rich waters streaming out of deep-sea hydrothermal vents. Evidence for a hot start included studies on the tree of life, which suggested that the most primitive species of microbes alive today thrive in hot water. But the hot-start hypothesis has cooled off a bit. Recent studies suggest that heat-loving microbes are not living fossils. Instead, they may have descended from less hardy species and evolved new defenses against heat. Some skeptics also wonder how delicate RNA molecules could have survived in boiling water. No single strong hypothesis has taken the hot start's place, however, although suggestions include tidal pools or oceans covered by glaciers.

Research projects now under way may shed more light on how life began. Scientists are running experiments in which RNA-based cells may be able to reproduce and evolve. NASA and the European Space Agency have launched probes that will visit comets, narrowing down the possible ingredients that might have been showered on early Earth.

Most exciting of all is the possibility of finding signs of life on Mars. Recent missions to Mars have provided strong evidence that shallow seas of liquid water once existed on the Red Planet—suggesting that Mars might once have been hospitable to life. Future Mars missions will look for signs of life hiding in underground refuges, or fossils of extinct creatures. If life does turn up, the discovery could mean that life arose independently on both planets—suggesting that it is common in the universe—or that it arose on one planet and spread to the other. Perhaps martian microbes were carried to Earth on a meteorite 4 billion years ago, infecting our sterile planet. —CARL ZIMMER

Carl Zimmer is the author of *Soul Made Flesh: The Discovery of the Brain—and How it Changed the World*.

How do organs and whole organisms know when to stop growing?
A person's right and left legs almost always end up the same length, and the hearts of mice and elephants each fit the proper rib cage. How genes set limits on cell size and number continues to mystify.

JUPITER IMAGES

How can genome changes other than mutations be inherited?
Researchers are finding ever more examples of this process, called epigenetics, but they can't explain what causes and preserves the changes.

How is asymmetry determined in the embryo?
Whirling cilia help an embryo tell its left from its right, but scientists are still looking for the first factors that give a relatively uniform ball of cells a head, tail, front, and back.

CENTER FOR FUNCTIONAL GENOMICS, SUNY AT ALBANY

continued >>

How do limbs, fins, and faces develop and evolve?
The genes that determine the length of a nose or the breadth of a wing are subject to natural and sexual selection. Understanding how selection works could lead to new ideas about the mechanics of evolution with respect to development.

What Determines Species Diversity

Countless species of plants, animals, and microbes fill every crack and crevice on land and in the sea. They make the world go 'round, converting sunlight to energy that fuels the rest of life, cycling carbon and nitrogen between inorganic and organic forms, and modifying the landscape.

In some places and some groups, hundreds of species exist, whereas in others, very few have evolved; the tropics, for example, are a complex paradise compared to higher latitudes. Biologists are striving to understand why. The interplay between environment and living organisms and between the organisms themselves play key roles in encouraging or discouraging diversity, as do human disturbances, predator-prey relationships, and other food web connections. But exactly how these and other forces work together to shape diversity is largely a mystery.

The challenge is daunting. Baseline data are poor, for example: We don't yet know how many plant and animal species there are on Earth, and researchers can't even begin to predict the numbers and kinds of organisms that make up the microbial world. Researchers probing the evolution of, and limits to, diversity also lack a standardized time scale because evolution takes place over periods lasting from days to millions of years. Moreover, there can be almost as much variation within a species as between two closely related ones. Nor is it clear what genetic changes will result in a new species and what their true influence on speciation is.

Understanding what shapes diversity will require a major interdisciplinary effort, involving paleontological interpretation, field studies, laboratory experimentation, genomic comparisons, and effective statistical analyses. A few exhaustive inventories, such as the United Nations' Millennium Project and an around-the-world assessment of genes from marine microbes, should improve baseline data, but they will barely scratch the surface. Models that predict when one species will split into two will help. And an emerging discipline called evo-devo is probing how genes involved in development contribute to evolution. Together, these efforts will go a long way toward clarifying the history of life.

Paleontologists have already made headway in tracking the expansion and contraction of the ranges of various organisms over the millennia. They are finding that geographic distribution plays a key role in speciation. Future studies should continue to reveal large-scale patterns of distribution and perhaps shed more light on the origins of mass extinctions and the effects of these catastrophes on the evolution of new species.

From field studies of plants and animals, researchers have learned that habitat can influence morphology and behavior—particularly sexual selection—in ways that hasten or slow down speciation. Evolutionary biologists have also discovered that speciation can stall out, for example, as separated populations become reconnected, homogenizing genomes that would otherwise diverge. Molecular forces, such as low mutation rates or meiotic drive—in which certain alleles have an increased likelihood of being passed from one generation to the next—influence the rate of speciation.

And in some cases, differences in diversity can vary within an ecosystem: Edges of ecosystems sometimes support fewer species than the interior.

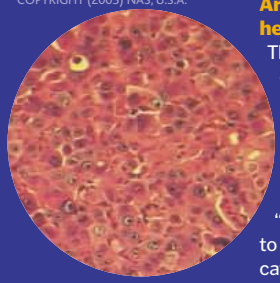
Evolutionary biologists are just beginning to sort out how all these factors are intertwined in different ways for different groups of organisms. The task is urgent: Figuring out what shapes diversity could be important for understanding the nature of the wave of extinctions the world is experiencing and for determining strategies to mitigate it.

—ELIZABETH PENNISI

CREDITS (TOP TO BOTTOM): STEPHEN FRINK/CORBIS IMAGES; KEN LUCAS/VISUALS UNLIMITED; HANS PFLETSCHINGER/PETER ARNOLD

What triggers puberty?
Nutrition—including that received in utero—seems to help set this mysterious biological clock, but no one knows exactly what forces childhood to end.

M. CLARKE *ET AL.*, *PNAS* **100** (7), 3983 COPYRIGHT (2003) NAS, U.S.A.



Are stem cells at the heart of all cancers?
The most aggressive cancer cells look a lot like stem cells. If cancers are caused by stem cells gone awry, studies of a cell's "stemness" may lead to tools that could catch tumors sooner and destroy them more effectively.

Is cancer susceptible to immune control?
Although our immune responses can suppress tumor growth, tumor cells can combat those responses with countermeasures. This defense can stymie researchers hoping to develop immune therapies against cancer.

L. HEUSER AND R. ACKLAND/UNIVERSITY OF LOUISVILLE SCHOOL OF MEDICINE

Can cancers be controlled rather than cured?
Drugs that cut off a tumor's fuel supplies—say, by stopping blood-vessel growth—can safely check or even reverse tumor growth. But how long the drugs remain effective is still unknown.

What Genetic Changes Made Us Uniquely Human

Every generation of anthropologists sets out to explore what it is that makes us human. Famed paleoanthropologist Louis Leakey thought tools made the man, and so when he uncovered hominid bones near stone tools in Tanzania in the 1960s, he labeled the putative toolmaker *Homo habilis*, the earliest member of the human genus. But then primatologist Jane Goodall demonstrated that chimps also use tools of a sort, and today researchers debate whether *H. habilis* truly belongs in *Homo*. Later studies have honed in on traits such as bipedality, culture, language, humor, and, of course, a big brain as the unique birthright of our species. Yet many of these traits can also be found, at least to some degree, in other creatures: Chimps have rudimentary culture, parrots speak, and some rats seem to giggle when tickled.

What is beyond doubt is that humans, like every other species, have a unique genome shaped by our evolutionary history. Now, for the first time, scientists can address anthropology's fundamental question at a new level: What are the genetic changes that make us human?

With the human genome in hand and primate genome data beginning to pour in, we are entering an era in which it may become possible to pinpoint the genetic changes that help separate us from our closest relatives. A rough draft of the chimp sequence has already been released, and a more detailed version is expected soon. The genome of the macaque is nearly complete, the orangutan is under way, and the marmoset was recently approved. All these will help reveal the ancestral genotype at key places on the primate tree.

The genetic differences revealed between humans and chimps are likely to be profound, despite the oft-repeated statistic that only about 1.2% of our DNA differs from that of chimps. A change in every 100th base could affect thousands of genes, and the percentage difference becomes much larger if you count insertions and deletions. Even if we document all of the perhaps 40 million sequence differences between humans and chimps, what do they mean? Many are probably simply the consequence of 6 million years of genetic drift, with little effect on body or behavior, whereas other small changes—perhaps in regulatory, noncoding sequences—may have dramatic consequences.

Half of the differences might define a chimp rather than a human. How can we sort them all out?

One way is to zero in on the genes that have been favored by natural selection in humans. Studies seeking subtle signs of selection in the DNA of humans and other primates have identified dozens of genes, in particular those involved in host-pathogen interactions, reproduction, sensory systems such as olfaction and taste, and more.

But not all of these genes helped set us apart from our ape cousins originally. Our genomes reveal that we have evolved in response to malaria, but malaria defense didn't make us human. So some researchers have started with clinical mutations that impair key traits, then traced the genes' evolution, an approach that has identified a handful of tantalizing genes. For example, *MCPH1* and *ASPM* cause microcephaly when mutated, *FOXP2* causes speech defects, and all three show signs of selection pressure during human, but not chimp, evolution. Thus they may have played roles in the evolution of humans' large brains and speech.

But even with genes like these, it is often difficult to be completely sure of what they do. Knockout experiments, the classic way to reveal function, can't be done in humans and apes for ethical reasons. Much of the work will therefore demand comparative analyses of the genomes and phenotypes of large numbers of humans and apes. Already, some researchers are pushing for a "great ape 'phenome' project" to match the incoming tide of genomic data with more phenotypic information on apes. Other researchers argue that clues to function can best be gleaned by mining natural human variability, matching mutations in living people to subtle differences in biology and behavior. Both strategies face logistical and ethical problems, but some progress seems likely.

A complete understanding of uniquely human traits will, however, include more than DNA. Scientists may eventually circle back to those long-debated traits of sophisticated language, culture, and technology, in which nurture as well as nature plays a leading role. We're in the age of the genome, but we can still recognize that it takes much more than genes to make the human.

—ELIZABETH CULOTTA

CREDITS (LEFT TO RIGHT): FRITZ POLKING/VISUALS UNLIMITED; TERRY HUSEBYE/GETTY IMAGES

Is inflammation a major factor in all chronic diseases?
It's a driver of arthritis, but cancer and heart disease? More and more, the answer seems to be yes, and the question remains why and how.

ART DAVIS/USDA

How do prion diseases work?
Even if one accepts that prions are just misfolded proteins, many mysteries remain. How can they go from the gut to the brain, and how do they kill cells once there, for example.

How much do vertebrates depend on the innate immune system to fight infection?
This system predates the vertebrate adaptive immune response. Its relative importance is unclear, but immunologists are working to find out.

JUPITER IMAGES

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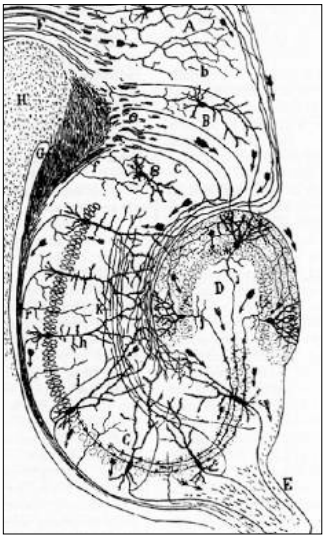
Does immunologic memory require chronic exposure to antigens?
Yes, say a few prominent thinkers, but experiments with mice now challenge the theory. Putting the debate to rest would require proving that something is not there, so the question likely will not go away.

How Are Memories Stored and Retrieved

Packed into the kilogram or so of neural wetware between the ears is everything we know: a compendium of useful and trivial facts about the world, the history of our lives, plus every skill we've ever learned, from riding a bike to persuading a loved one to take out the trash. Memories make each of us unique, and they give continuity to our lives. Understanding how memories are stored in the brain is an essential step toward understanding ourselves.

Neuroscientists have already made great strides, identifying key brain regions and potential molecular mechanisms. Still, many important questions remain unanswered, and a chasm gapes between the molecular and whole-brain research.

The birth of the modern era of memory research is often pegged to the publication, in 1957, of an account of the neurological patient H.M. At age 27, H.M. had large chunks of the temporal lobes of his brain surgically removed in a last-ditch effort to relieve chronic epilepsy. The surgery worked, but it left H.M. unable to remember anything that happened—or anyone he met—after his surgery. The case showed that the medial temporal lobes (MTL), which include the hippocampus, are crucial for making new memories. H.M.'s case also revealed, on closer examination, that memory is not a monolith: Given a tricky mirror drawing task, H.M.'s performance improved steadily over 3 days even though he had no memory of his previous practice. Remembering how is not the same as remembering what, as far as the brain is concerned.



Memorable diagram. Santiago Ramón y Cajal's drawing of the hippocampus. He proposed that memories involve strengthened neural connections.

Thanks to experiments on animals and the advent of human brain imaging, scientists now have a working knowledge of the various kinds of memory as well as which parts of the brain are involved in each. But persistent gaps remain. Although the MTL has indeed proved critical for declarative memory—the recollection of facts and events—the region remains something of a black box. How its various components interact during memory encoding and retrieval is unresolved. Moreover, the MTL is not the final repository of declarative memories. Such memories are apparently filed to the cerebral cortex for long-term storage, but how this happens, and how memories are represented in the cortex, remains unclear.

More than a century ago, the great Spanish neuroanatomist Santiago Ramón y Cajal proposed that making memories must require neurons to strengthen their connections with one another. Dogma at the time held that no new neurons are born in the adult brain, so Ramón y Cajal made the reasonable assumption that the key changes must occur between existing neurons. Until recently, scientists had few clues about how this might happen.

Since the 1970s, however, work on isolated chunks of nervous-system tissue has identified a host of molecular players in memory formation. Many of the same molecules have been implicated in both declarative and nondeclarative memory and in species as varied as sea slugs, fruit flies, and rodents, suggesting that the molecular machinery for memory has been widely conserved. A key insight from this work has been that short-term memory (lasting minutes) involves chemical modifications that strengthen existing connections, called synapses, between neurons, whereas long-term memory (lasting days or weeks) requires protein synthesis and probably the construction of new synapses.

Tying this work to the whole-brain research is a major challenge. A potential bridge is a process called long-term potentiation (LTP), a type of synaptic strengthening that has been scrutinized in slices of rodent hippocampus and is widely considered a likely physiological basis for memory. A conclusive demonstration that LTP really does underlie memory formation in vivo would be a big breakthrough.

Meanwhile, more questions keep popping up. Recent studies have found that patterns of neural activity seen when an animal is learning a new task are replayed later during sleep. Could this play a role in solidifying memories? Other work shows that our memories are not as trustworthy as we generally assume. Why is memory so labile? A hint may come from recent studies that revive the controversial notion that memories are briefly vulnerable to manipulation each time they're recalled. Finally, the no-new-neurons dogma went down in flames in the 1990s, with the demonstration that the hippocampus, of all places, is a virtual neuron nursery throughout life. The extent to which these newborn cells support learning and memory remains to be seen.

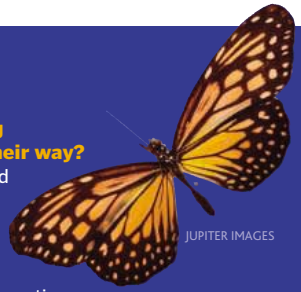
—GREG MILLER

Why doesn't a pregnant woman reject her fetus?
Recent evidence suggests that the mother's immune system doesn't "realize" that the fetus is foreign even though it gets half its genes from the father. Yet just as Nobelist Peter Medawar said when he first raised this question in 1952, "the verdict has yet to be returned."

JUPITER IMAGES

What synchronizes an organism's circadian clocks?
Circadian clock genes have popped up in all types of creatures and in many parts of the body. Now the challenge is figuring out how all the gears fit together and what keeps the clocks set to the same time.

How do migrating organisms find their way?
Birds, butterflies, and whales make annual journeys of thousands of kilometers. They rely on cues such as stars and magnetic fields, but the details remain unclear.



JUPITER IMAGES

Why do we sleep?
A sound slumber may refresh muscles and organs or keep animals safe from dangers lurking in the dark. But the real secret of sleep probably resides in the brain, which is anything but still while we're snoring away.

How Did Cooperative Behavior Evolve

When Charles Darwin was working out his grand theory on the origin of species, he was perplexed by the fact that animals from ants to people form social groups in which most individuals work for the common good. This seemed to run counter to his proposal that individual fitness was key to surviving over the long term.

By the time he wrote *The Descent of Man*, however, he had come up with a few explanations. He suggested that natural selection could encourage altruistic behavior among kin so as to improve the reproductive potential of the "family." He also introduced the idea of reciprocity: that unrelated but familiar individuals would help each other out if both were altruistic. A century of work with dozens of social species has borne out his ideas to some degree, but the details of how and why cooperation evolved remain to be worked out. The answers could help explain human behaviors that seem to make little sense from a strict evolutionary perspective, such as risking one's life to save a drowning stranger.

Animals help each other out in many ways. In social species from honeybees to naked mole rats, kinship fosters cooperation: Females forgo reproduction and instead help the dominant female with her young. And common agendas help unrelated individuals work together. Male chimpanzees, for example, gang up against predators, protecting each other at a potential cost to themselves.

Generosity is pervasive among humans. Indeed, some anthropologists argue that the evolution of the tendency to trust one's relatives and neighbors helped humans become Earth's dominant vertebrate: The ability to work together provided our early ancestors more food, better protection, and better childcare, which in turn improved reproductive success.

However, the degree of cooperation varies. "Cheaters" can gain a leg up on the rest of humankind, at least in the short term. But cooperation prevails among many species, suggesting that this behavior is a better survival strategy, over the long run, despite all the strife among ethnic, political, religious, even family groups now rampant within our species.

Evolutionary biologists and animal behavior researchers are searching out the genetic basis and molecular drivers of cooperative behaviors, as well as the physiological, environmental, and behavioral impetus for sociality. Neuroscientists studying mammals from voles to hyenas are discovering key correlations between brain chemicals and social strategies.

Others with a more mathematical bent are applying evolutionary game theory, a modeling approach developed for economics, to quantify cooperation and predict behavioral outcomes under different circumstances. Game theory has helped reveal a seemingly innate desire for fairness: Game players will spend time and energy to punish unfair actions, even though there's nothing to be gained by these actions for themselves. Similar studies have shown that even when two people meet just once, they tend to be fair to each other. Those actions are hard to explain, as they don't seem to follow the basic tenet that cooperation is really based on self-interest.

The models developed through these games are still imperfect. They do not adequately consider, for example, the effect of emotions on cooperation. Nonetheless, with game theory's increasing sophistication, researchers hope to gain a clearer sense of the rules that govern complex societies.

Together, these efforts are helping social scientists and others build on Darwin's observations about cooperation. As Darwin predicted, reciprocity is a powerful fitness tactic. But it is not a pervasive one.

Modern researchers have discovered that a good memory is a prerequisite: It seems reciprocity is practiced only by organisms that can keep track of those who are helpful and those who are not. Humans have a great memory for faces and thus can maintain lifelong good—or hard—feelings toward people they don't see for years. Most other species exhibit reciprocity only over very short time scales, if at all.

Limited to his personal observations, Darwin was able to come up with only general rationales for cooperative behavior. Now, with new insights from game theory and other promising experimental approaches, biologists are refining Darwin's ideas and, bit by bit, hope that one day they will understand just what it takes to bring out our cooperative spirit.

—ELIZABETH PENNISI

CREDITS (TOP TO BOTTOM): TOM BRAKEFIELD/CORBIS; M. THOMSEN/CORBIS

Why do we dream?
Freud thought dreaming provides an outlet for our unconscious desires. Now, neuroscientists suspect that brain activity during REM sleep—when dreams occur—is crucial for learning. Is the experience of dreaming just a side effect?

JUPITER IMAGES

Why are there critical periods for language learning?
Monitoring brain activity in young children—including infants—may shed light on why children pick up languages with ease while adults often struggle to learn train station basics in a foreign tongue.

Do pheromones influence human behavior?
Many animals use airborne chemicals to communicate, particularly when mating. Controversial studies have hinted that humans too use pheromones. Identifying them will be key to assessing their sway on our social lives.

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How do general anesthetics work?
Scientists are chipping away at the drugs' effects on individual neurons, but understanding how they render us unconscious will be a tougher nut to crack.

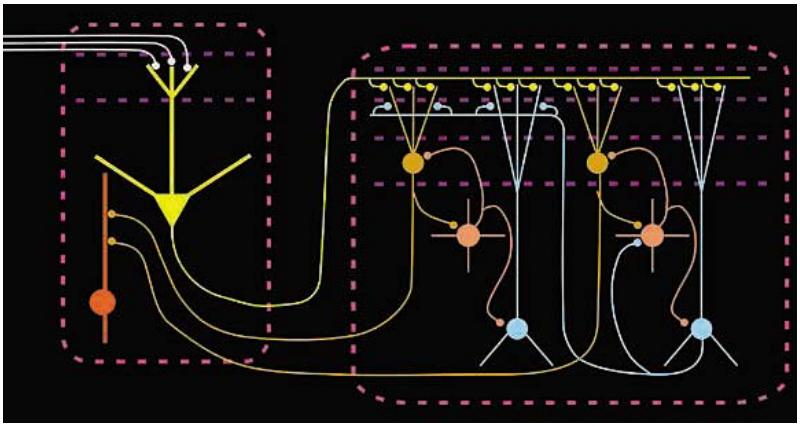
How Will Big Pictures Emerge From a Sea of Biological Data

Biology is rich in descriptive data—and getting richer all the time. Large-scale methods of probing samples, such as DNA sequencing, microarrays, and automated gene-function studies, are filling new databases to the brim. Many subfields from biomechanics to ecology have gone digital, and as a result, observations are more precise and more plentiful. A central question now confronting virtually all fields of biology is whether scientists can deduce from this torrent of molecular data how systems and whole organisms work. All this information needs to be sifted, organized, compiled, and—most importantly—connected in a way that enables researchers to make predictions based on general principles.

Enter systems biology. Loosely defined and still struggling to find its way, this newly emerging approach aims to connect the dots that have emerged from decades of molecular, cellular, organismal, and even environmental observations. Its proponents seek to make biology more quantitative by relying on mathematics, engineering, and computer science to build a more rigid framework for linking disparate findings. They argue that it is the only way the field can move forward. And they suggest that biomedicine, particularly deciphering risk factors for disease, will benefit greatly.

The field got a big boost from the completion of the human genome sequence. The product of a massive, trip-to-the-moon logistical effort, the sequence is now a hard and fast fact. The biochemistry of human inheritance has been defined and measured. And that has inspired researchers to try to make other aspects of life equally knowable.

Molecular geneticists dream of having a similarly comprehensive view of networks that control genes: For example, they would like to identify rules explaining how a single DNA sequence can express different proteins, or varying amounts of protein, in different circumstances (see p. 80). Cell biologists would like to reduce the complex communication patterns traced by molecules that regulate the health of the cell to a set of signaling rules. Developmental biologists would like a comprehensive picture of how the embryo manages to direct a handful of cells into a myriad of specialized functions in bone, blood, and skin tissue. These hard puzzles can only be solved by systems biology, proponents say.



Systems approach. Circuit diagrams help clarify nerve cell functions.

The same can be said for neuroscientists trying to work out the emergent properties—higher thought, for example—hidden in complex brain circuits. To understand ecosystem changes, including global warming, ecologists need ways to incorporate physical as well as biological data into their thinking.

Today, systems biologists have only begun to tackle relatively simple networks. They have worked out the metabolic pathway in yeast for breaking down galactose, a carbohydrate. Others have tracked the first few hours of the embryonic development of sea urchins and other organisms with the goal of seeing how various transcription factors alter gene expression over time. Researchers are also developing rudimentary models of signaling networks in cells and simple brain circuits.

Progress is limited by the difficulty of translating biological patterns into computer models. Network computer programs themselves are relatively simple, and the methods of portraying the results in ways that researchers can understand and interpret need improving. New institutions around the world are gathering interdisciplinary teams of biologists, mathematicians, and computer specialists to help promote systems biology approaches. But it is still in its early days.

No one yet knows whether intensive interdisciplinary work and improved computational power will enable researchers to create a comprehensive, highly structured picture of how life works.

—ELIZABETH PENNISI

CREDIT: MICHAEL T. SHIPLEY

What causes schizophrenia?
Researchers are trying to track down genes involved in this disorder. Clues may also come from research on traits schizophrenics share with normal people.

What causes autism?
Many genes probably contribute to this baffling disorder, as well as unknown environmental factors. A biomarker for early diagnosis would help improve existing therapy, but a cure is a distant hope.

To what extent can we stave off Alzheimer's?
A 5- to 10-year delay in this late-onset disease would improve old age for millions. Researchers are determining whether treatments with hormones or antioxidants, or mental and physical exercise, will help.

ROYALTY-FREE/CORBIS

What is the biological basis of addiction?
Addiction involves the disruption of the brain's reward circuitry. But personality traits such as impulsivity and sensation-seeking also play a part in this complex behavior.

How Far Can We Push Chemical Self-Assembly

Most physical scientists nowadays focus on uncovering nature's mysteries; chemists build things. There is no synthetic astronomy or synthetic physics, at least for now. But chemists thrive on finding creative new ways to assemble molecules. For the last 100 years, they have done that mostly by making and breaking the strong covalent bonds that form when atoms share electrons. Using that trick, they have learned to combine as many as 1000 atoms into essentially any molecular configuration they please.

Impressive as it is, this level of complexity pales in comparison to what nature flaunts all around us. Everything from cells to cedar trees is knit together using a myriad of weaker links between small molecules. These weak interactions, such as hydrogen bonds, van der Waals forces, and π–π interactions, govern the assembly of everything from DNA in its famous double helix to the bonding of H_2O molecules in liquid water. More than just riding herd on molecules, such subtle forces make it possible for structures to assemble themselves into an ever more complex hierarchy. Lipids coalesce to form cell membranes. Cells organize to form tissues. Tissues combine to create organisms. Today, chemists can't approach the complexity of what nature makes look routine. Will they ever learn to make complex structures that self-assemble?

Well, they've made a start. Over the past 3 decades, chemists have made key strides in learning the fundamental rules of noncovalent bonding. Among these rules: *Like prefers like*. We see this in hydrophobic and hydrophilic interactions that propel lipid molecules in water to corral together to form the two-layer membranes that serve as the coatings surrounding cells. They bunch their oily tails together to avoid any interaction with water and leave their more polar head groups facing out into the liquid. Another rule: *Self-assembly is governed by energetically favorable reactions*. Leave the right component molecules alone, and they will assemble themselves into complex ordered structures.

Chemists have learned to take advantage of these and other rules to design self-assembling systems with a modest degree of complexity. Drug-carrying liposomes, made with lipid bilayers resembling those in cells, are used commercially to ferry drugs to cancerous tissues in patients. And self-assembled molecules called rotaxanes, which can act as molecular switches that oscillate back and forth between two stable states, hold promise as switches in future molecular-based computers.

But the need for increased complexity is growing, driven by the miniaturization of computer circuitry and the rise of nanotechnology. As features on computer chips continue to shrink, the cost of manufacturing these ever-smaller components is skyrocketing. Right now, companies make them by whittling materials down to the desired size. At some point, however, it will become cheaper to design and build them chemically from the bottom up.

Self-assembly is also the only practical approach for building a wide variety of nanostructures. Making sure the components assemble themselves correctly, however, is not an easy task. Because the forces at work are so small, self-assembling molecules can get trapped in undesirable conformations, making defects all but impossible to avoid. Any new system that relies on self-assembly must be able either to tolerate those defects or repair them. Again, biology offers an example in DNA. When enzymes copy DNA strands during cell division, they invariably make mistakes—occasionally inserting an A when they should have inserted a T, for example. Some of those mistakes get by, but most are caught by DNA-repair enzymes that scan the newly synthesized strands and correct copying errors.

Strategies like that won't be easy for chemists to emulate. But if they want to make complex, ordered structures from the ground up, they'll have to get used to thinking a bit more like nature.

–ROBERT F. SERVICE

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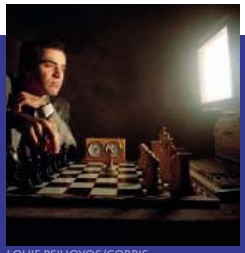

LOUIE PSIHOYOS/CORBIS

Is morality hard-wired into the brain?
That question has long puzzled philosophers; now some neuroscientists think brain imaging will reveal circuits involved in reasoning.

What are the limits of learning by machines?
Computers can already beat the world's best chess players, and they have a wealth of information on the Web to draw on. But abstract reasoning is still beyond any machine.

How much of personality is genetic?
Aspects of personality are influenced by genes; environment modifies the genetic effects. The relative contributions remain under debate.

JUPITER IMAGES

What is the biological root of sexual orientation?
Much of the "environmental" contribution to homosexuality may occur before birth in the form of prenatal hormones, so answering this question will require more than just the hunt for "gay genes."

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What Are the Limits of Conventional Computing

At first glance, the ultimate limit of computation seems to be an engineering issue. How much energy can you put in a chip without melting it? How fast can you flip a bit in your silicon memory? How big can you make your computer and still fit it in a room? These questions don't seem terribly profound.

In fact, computation is more abstract and fundamental than figuring out the best way to build a computer. This realization came in the mid-1930s, when Princeton mathematicians Alonzo Church and Alan Turing showed—roughly speaking—that any calculation involving bits and bytes can be done on an idealized computer known as a Turing machine. By showing that all classical computers are essentially alike, this discovery enabled scientists and mathematicians to ask fundamental questions about computation without getting bogged down in the minutiae of computer architecture.

For example, theorists can now classify computational problems into broad categories. *P* problems are those, broadly speaking, that can be solved quickly, such as alphabetizing a list of names. *NP* problems are much tougher to solve but relatively easy to check once you've reached an answer. An example is the traveling salesman problem, finding the shortest possible route through a series of locations. All known algorithms for getting an answer take lots of computing power, and even relatively small versions might be out of reach of any classical computer.

Mathematicians have shown that if you could come up with a quick and easy shortcut to solving any one of the hardest type of *NP* problems, you'd be able to crack them all. In effect, the *NP* problems would turn into *P* problems. But it's uncertain whether such a shortcut exists—whether $P = NP$. Scientists think not, but proving this is one of the great unanswered questions in mathematics.

In the 1940s, Bell Labs scientist Claude Shannon showed that bits are not just for computers; they are the fundamental units of describing the information that flows from one object to another. There are physical laws that govern how fast a bit can move from place to place, how much information can be transferred back and forth over a given communications channel, and how much energy it takes to erase a bit from memory. All classical information-processing machines are subject to these laws—and because information seems to be rattling back and forth in our brains, do the laws of information mean that our thoughts are reducible to bits and bytes? Are we merely computers? It's an unsettling thought.

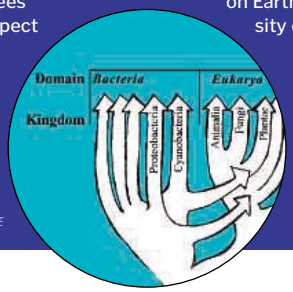
But there is a realm beyond the classical computer: the quantum. The probabilistic nature of quantum theory allows atoms and other quantum objects to store information that's not restricted to only the binary 0 or 1 of information theory, but can also be 0 and 1 at the same time. Physicists around the world are building rudimentary quantum computers that exploit this and other quantum effects to do things that are provably impossible for ordinary computers, such as finding a target record in a database with too few queries. But scientists are still trying to figure out what quantum-mechanical properties make quantum computers so powerful and to engineer quantum computers big enough to do something useful.

By learning the strange logic of the quantum world and using it to do computing, scientists are delving deep into the laws of the subatomic world. Perhaps something as seemingly mundane as the quest for computing power might lead to a newfound understanding of the quantum realm.

—CHARLES SEIFE

CREDIT: JUPITER IMAGES

Will there ever be a tree of life that systematists can agree on?
Despite better morphological, molecular, and statistical methods, researchers' trees don't agree. Expect greater, but not complete, consensus.



W. F. DOOLITTLE, *SCIENCE* **284**, 2124 (1999)

How many species are there on Earth?
Count all the stars in the sky? Impossible. Count all the species on Earth? Ditto. But the biodiversity crisis demands that we try.

What is a species?
A "simple" concept that's been muddied by evolutionary data; a clear definition may be a long time in coming.

Why does lateral transfer occur in so many species and how?
Once considered rare, gene swapping, particularly among microbes, is proving quite common. But why and how genes are so mobile—and the effect on fitness—remains to be determined.

M. DWORKIN, *MICROBIOL. REVS.* **60**, 70 (1996)

Who was LUCA (the last universal common ancestor)?
Ideas about the origin of the 1.5-billion-year-old "mother" of all complex organisms abound. The continued discovery of primitive microbes, along with comparative genomics, should help resolve life's deep past.

Can We Selectively Shut Off Immune Responses

In the past few decades, organ transplantation has gone from experimental to routine. In the United States alone, more than 20,000 heart, liver, and kidney transplants are performed every year. But for transplant recipients, one prospect has remained unchanged: a lifetime of taking powerful drugs to suppress the immune system, a treatment that can have serious side effects. Researchers have long sought ways to induce the immune system to tolerate a transplant without blunting the body's entire defenses, but so far, they have had limited success.

They face formidable challenges. Although immune tolerance can occur—in rare cases, transplant recipients who stop taking immunosuppressants have not rejected their foreign organs—researchers don't have a clear picture of what is happening at the molecular and cellular levels to allow this to happen. Tinkering with the immune system is also a bit like tinkering with a mechanical watch: Fiddle with one part, and you may disrupt the whole mechanism. And there is a big roadblock to testing drugs designed to induce tolerance: It is hard to know if they work unless immunosuppressant drugs are withdrawn, and that would risk rejection of the transplant. But if researchers can figure out how to train the immune system to tolerate transplants, the knowledge could have implications for the treatment of autoimmune diseases, which also result from unwanted immune attack—in these cases on some of the body's own tissues.

A report in *Science* 60 years ago fired the starting gun in the race to induce transplant tolerance—a race that has turned into a marathon. Ray Owen of the University of Wisconsin, Madison, reported that fraternal twin cattle sometimes share a placenta and are born with each other's red blood cells, a state referred to as mixed chimerism. The cattle tolerated the foreign cells with no apparent problems.

A few years later, Peter Medawar and his team at the University of Birmingham, U.K., showed that fraternal twin cattle with mixed chimerism readily accept skin grafts from each other. Medawar did not immediately appreciate the link to Owen's work, but when he saw the connection, he decided to inject fetal mice in utero with tissue from mice of a different strain. In a publication in *Nature* in 1953, the researchers showed that, after birth, some of these mice tolerated skin grafts from different strains. This influential experiment led many to devote their careers to transplantation and also raised hopes that the work would lead to cures for autoimmune diseases.

Immunologists, many of them working with mice, have since spelled out several detailed mechanisms behind tolerance. The immune system can, for example, dispatch "regulatory" cells that suppress immune attacks against self. Or the system can force harmful immune cells to commit suicide or to go into a dysfunctional stupor called anergy. Researchers indeed now know fine details about the genes, receptors, and cell-to-cell communications that drive these processes.

CREDIT: MARK HARMEL/GETTY IMAGES

Yet it's one matter to unravel how the immune system works and another to figure out safe ways to manipulate it. Transplant researchers are pursuing three main strategies to induce tolerance. One builds on Medawar's experiments by trying to exploit chimerism. Researchers infuse the patient with the organ donor's bone marrow in hopes that the donor's immune cells will teach the host to tolerate the transplant; donor immune cells that come along with the transplanted organ also, some contend, can teach tolerance. A second strategy uses drugs to train T cells to become anergic or commit suicide when they see the foreign antigens on the transplanted tissue. The third approach turns up production of T regulatory cells, which prevent specific immune cells from copying themselves and can also suppress rejection by secreting biochemicals called cytokines that direct the immune orchestra to change its tune.

All these strategies face a common problem: It is maddeningly difficult to judge whether the approach has failed or succeeded because there are no reliable "biomarkers" that indicate whether a person has become tolerant to a transplant. So the only way to assess tolerance is to stop drug treatment, which puts the patient at risk of rejecting the organ. Similarly, ethical concerns often require researchers to test drugs aimed at inducing tolerance in concert with immunosuppressive therapy. This, in turn, can undermine the drugs' effectiveness because they need a fully functioning immune system to do their job.

If researchers can complete their 50-year quest to induce immune tolerance safely and selectively, the prospects for hundreds of thousands of transplant recipients would be greatly improved, and so, too, might the prospects for controlling autoimmune diseases.

–JON COHEN

How did flowers evolve?
Darwin called this question an "abominable mystery." Flowers arose in the cycads and conifers, but the details of their evolution remain obscure.

How do plants make cell walls?
Cellulose and pectin walls surround cells, keeping water in and supporting tall trees. The biochemistry holds the secrets to turning its biomass into fuel.

J. MOGLIA/*SCIENCE*

How is plant growth controlled?
Redwoods grow to be hundreds of meters tall, Arctic willows barely 10 centimeters. Understanding the difference could lead to higher-yielding crops.

Why aren't all plants immune to all diseases?
Plants can mount a general immune response, but they also maintain molecular snipers that take out specific pathogens. Plant pathologists are asking why different species, even closely related ones, have different sets of defenders. The answer could result in hardier crops.

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What is the basis of variation in stress tolerance in plants?
We need crops that better withstand drought, cold, and other stresses. But there are so many genes involved, in complex interactions, that no one has yet figured out which ones work how.

Do Deeper Principles Underlie Quantum Uncertainty and Nonlocality

"Quantum mechanics is very impressive," Albert Einstein wrote in 1926. "But an inner voice tells me that it is not yet the real thing." As quantum theory matured over the years, that voice has gotten quieter—but it has not been silenced. There is a relentless murmur of confusion underneath the chorus of praise for quantum theory.

Quantum theory was born at the very end of the 19th century and soon became one of the pillars of modern physics. It describes, with incredible precision, the bizarre and counterintuitive behavior of the very small: atoms and electrons and other wee beasties of the submicroscopic world. But that success came with the price of discomfort. The equations of quantum mechanics work very well; they just don't seem to make sense.

No matter how you look at the equations of quantum theory, they allow a tiny object to behave in ways that defy intuition. For example, such an object can be in "superposition": It can have two mutually exclusive properties at the same time. The mathematics of quantum theory says that an atom, for example, can be on the left side of a box and the right side of the box at the very same instant, as long as the atom is undisturbed and unobserved. But as soon as an observer opens the box and tries to spot where the atom is, the superposition collapses and the atom instantly "chooses" whether to be on the right or the left.

This idea is almost as unsettling today as it was 80 years ago, when Erwin Schrödinger ridiculed superposition by describing a half-living, half-dead cat. That is because quantum theory changes what the meaning of "is" is. In the classical world, an object has a solid reality: Even a cloud of gas is well described by hard little billiard ball–like pieces, each of which has a well-defined position and velocity. Quantum theory seems to undermine that solid reality. Indeed, the famous Uncertainty Principle, which arises directly from the mathematics of quantum theory, says that objects' positions and momenta are smeary and ill defined, and gaining knowledge about one implies losing knowledge about the other.

The early quantum physicists dealt with this unreality by saying that the "is"—the fundamental objects handled by the equations of quantum theory—were not actually particles that had an extrinsic reality but "probability waves" that merely had the capability of becoming "real" when an observer makes a measurement. This so-called Copenhagen Interpretation makes sense, if you're willing to accept that reality is probability waves and not solid objects. Even so, it still doesn't sufficiently explain another weirdness of quantum theory: nonlocality.

In 1935, Einstein came up with a scenario that still defies common sense. In his thought experiment, two particles fly away from each other and wind up at opposite ends of the galaxy. But the two particles happen to be "entangled"—linked in a quantum-mechanical sense—so that one particle instantly "feels" what happens to its twin. Measure one, and the other is instantly transformed by that measurement as well; it's as if the twins mystically communicate, instantly, over vast regions of space. This "nonlocality" is a mathematical consequence of quantum theory and has been measured in the lab. The spooky action apparently ignores distance and the flow of time; in theory, particles can be entangled *after* their entanglement has already been measured.

On one level, the weirdness of quantum theory isn't a problem at all. The mathematical framework is sound and describes all these bizarre phenomena well. If we humans can't imagine a physical reality that corresponds to our equations, so what? That attitude has been called the "shut up and calculate" interpretation of quantum mechanics. But to others, our difficulties in wrapping our heads around quantum theory hint at greater truths yet to be understood.

Some physicists in the second group are busy trying to design experiments that can get to the heart of the weirdness of quantum theory. They are slowly testing what causes quantum superpositions to "collapse"—research that may gain insight into the role of measurement in quantum theory as well as into why big objects behave so differently from small ones. Others are looking for ways to test various explanations for the weirdnesses of quantum theory, such as the "many worlds" interpretation, which explains superposition, entanglement, and other quantum phenomena by positing the existence of parallel universes. Through such efforts, scientists might hope to get beyond the discomfort that led Einstein to declare that "[God] does not play dice."

—CHARLES SEIFE

CREDIT: RIMMA GERLOVINA AND VALERIY GERLOVIN "TWO EGGS" © 2002, C-PRINT. COURTESY ROBERT BROWN GALLERY, WASHINGTON, D.C.

What caused mass extinctions?
A huge impact did in the dinosaurs, but the search for other catastrophic triggers of extinction has had no luck so far. If more subtle or stealthy culprits are to blame, they will take considerably longer to find.

Can we prevent extinction?
Finding cost-effective and politically feasible ways to save many endangered species requires creative thinking.

Why were some dinosaurs so large?
Dinosaurs reached almost unimaginable sizes, some in less than 20 years. But how did the long-necked sauropods, for instance, eat enough to pack on up to 100 tons without denuding their world?

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How will ecosystems respond to global warming?
To anticipate the effects of the intensifying greenhouse, climate modelers will have to focus on regional changes and ecologists on the right combination of environmental changes.

Is an Effective HIV Vaccine Feasible

In the 2 decades since researchers identified HIV as the cause of AIDS, more money has been spent on the search for a vaccine against the virus than on any vaccine effort in history. The U.S. National Institutes of Health alone invests nearly $500 million each year, and more than 50 different preparations have entered clinical trials. Yet an effective AIDS vaccine, which potentially could thwart millions of new HIV infections each year, remains a distant dream.

Although AIDS researchers have turned the virus inside-out and carefully detailed how it destroys the immune system, they have yet to unravel which immune responses can fend off an infection. That means, as one AIDS vaccine researcher famously put it more than a decade ago, the field is "flying without a compass."

Some skeptics contend that no vaccine will ever stop HIV. They argue that the virus replicates so quickly and makes so many mistakes during the process that vaccines can't possibly fend off all the types of HIV that exist. HIV also has developed sophisticated mechanisms to dodge immune attack, shrouding its surface protein in sugars to hide vulnerable sites from antibodies and producing proteins that thwart production of other immune warriors. And the skeptics point out that vaccine developers have had little success against pathogens like HIV that routinely outwit the immune system—the malaria parasite, hepatitis C virus, and the tuberculosis bacillus are prime examples.

Yet AIDS vaccine researchers have solid reasons to believe they can succeed. Monkey experiments have shown that vaccines can protect animals from SIV, a simian relative of HIV. Several studies have identified people who repeatedly expose themselves to HIV but remain uninfected, suggesting that something is stopping the virus. A small percentage of people who do become infected never seem to suffer any harm, and others hold the virus at bay for a decade or more before showing damage to their immune systems. Scientists also have found that some rare antibodies do work powerfully against the virus in test tube experiments.

At the start, researchers pinned their hopes on vaccines designed to trigger production of antibodies against HIV's surface protein. The approach seemed promising because HIV uses the surface protein to latch onto white blood cells and establish an infection. But vaccines that only contained HIV's surface protein looked lackluster in animal and test tube studies, and then proved worthless in large-scale clinical trials.

CREDIT: REUTERS/CORBIS

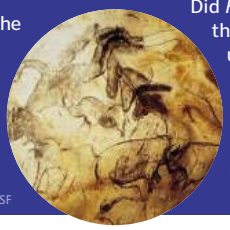
Now, researchers are intensely investigating other approaches. When HIV manages to thwart antibodies and establish an infection, a second line of defense, cellular immunity, specifically targets and eliminates HIV-infected cells. Several vaccines which are now being tested aim to stimulate production of killer cells, the storm troopers of the cellular immune system. But cellular immunity involves other players—such as macrophages, the network of chemical messengers called cytokines, and so-called natural killer cells—that have received scant attention.

The hunt for an antibody-based vaccine also is going through something of a renaissance, although it's requiring researchers to think backward. Vaccine researchers typically start with antigens—in this case, pieces of HIV—and then evaluate the antibodies they elicit. But now researchers have isolated more than a dozen antibodies from infected people that have blocked HIV infection in test tube experiments. The trick will be to figure out which specific antigens triggered their production.

It could well be that a successful AIDS vaccine will need to stimulate both the production of antibodies and cellular immunity, a strategy many are attempting to exploit. Perhaps the key will be stimulating immunity at mucosal surfaces, where HIV typically enters. It's even possible that researchers will discover an immune response that no one knows about today. Or perhaps the answer lies in the interplay between the immune system and human genetic variability: Studies have highlighted genes that strongly influence who is most susceptible—and who is most resistant—to HIV infection and disease.

Wherever the answer lies, the insights could help in the development of vaccines against other diseases that, like HIV, don't easily succumb to immune attack and that kill millions of people. Vaccine developers for these diseases will probably also have to look in unusual places for answers. The maps created by AIDS vaccine researchers currently exploring uncharted immunologic terrain could prove invaluable. –JON COHEN

How many kinds of humans coexisted in the recent past, and how did they relate?
The new dwarf human species fossil from Indonesia suggests that at least four kinds of humans thrived in the past 100,000 years. Better dates and additional material will help confirm or revise this picture.



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What gave rise to modern human behavior?
Did *Homo sapiens* acquire abstract thought, language, and art gradually or in a cultural "big bang," which in Europe occurred about 40,000 years ago? Data from Africa, where our species arose, may hold the key to the answer.

What are the roots of human culture?
No animal comes close to having humans' ability to build on previous discoveries and pass the improvements on. What determines those differences could help us understand how human culture evolved.

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What are the evolutionary roots of language and music?
Neuroscientists exploring how we speak and make music are just beginning to find clues as to how these prized abilities arose.

How Hot Will The Greenhouse World Be

Scientists know that the world has warmed lately, and they believe humankind is behind most of that warming. But how far might we push the planet in coming decades and centuries? That depends on just how sensitively the climate system—air, oceans, ice, land, and life—responds to the greenhouse gases we're pumping into the atmosphere. For a quarter-century, expert opinion was vague about climate sensitivity. Experts allowed that climate might be quite touchy, warming sharply when shoved by one climate driver or another, such as the carbon dioxide from fossil fuel burning, volcanic debris, or dimming of the sun. On the other hand, the same experts conceded that climate might be relatively unresponsive, warming only modestly despite a hard push toward the warm side.

A harbinger? Coffins being lined up during the record-breaking 2003 heat wave in Europe.

The problem with climate sensitivity is that you can't just go out and directly measure it. Sooner or later a climate model must enter the picture. Every model has its own sensitivity, but each is subject to all the uncertainties inherent in building a hugely simplified facsimile of the real-world climate system. As a result, climate scientists have long quoted the same vague range for sensitivity: A doubling of the greenhouse gas carbon dioxide, which is expected to occur this century, would eventually warm the world between a modest 1.5°C and a whopping 4.5°C. This range—based on just two early climate models—first appeared in 1979 and has been quoted by every major climate assessment since.

Researchers are finally beginning to tighten up the range of possible sensitivities, at least at one end. For one, the sensitivities of the available models (5% to 95% confidence range) are now falling within the canonical range of 1.5°C to 4.5°C; some had gone considerably beyond the high end. And the first try at a new approach—running a single model while varying a number of model parameters such as cloud behavior—has produced a sensitivity range of 2.4°C to 5.4°C with a most probable value of 3.2°C.

Models are only models, however. How much better if nature ran the experiment? Enter paleoclimatologists, who sort out how climate drivers such as greenhouse gases have varied naturally in the distant past and how the climate system of the time responded. Nature, of course, has never run the perfect analog for the coming greenhouse warming. And estimating how much carbon dioxide concentrations fell during the depths of the last ice age or how much sunlight debris from the eruption of Mount Pinatubo in the Philippines blocked will always have lingering uncertainties. But paleoclimate estimates of climate sensitivity generally fall in the canonical range, with a best estimate in the region of 3°C.

The lower end at least of likely climate sensitivity does seem to be firming up; it's not likely below 1.5°C, say researchers. That would rule out the negligible warmings proposed by some greenhouse contrarians. But climate sensitivity calculations still put a fuzzy boundary on the high end. Studies drawing on the past century's observed climate change plus estimates of natural and anthropogenic climate drivers yield up to 30% probabilities of sensitivities above 4.5°C, ranging as high as 9°C. The latest study that varies model parameters allows sensitivities up to 11°C, with the authors contending that they can't yet say what the chances of such extremes are. Others are pointing to times of extreme warmth in the geologic past that climate models fail to replicate, suggesting that there's a dangerous element to the climate system that the models do not yet contain.

Climate researchers have their work cut out for them. They must inject a better understanding of clouds and aerosols—the biggest sources of uncertainty—into their modeling. Ten or 15 years ago, scientists said that would take 10 or 15 years; there's no sign of it happening anytime soon. They must increase the fidelity of models, a realistic goal given the continued acceleration of affordable computing power. And they must retrieve more and better records of past climate changes and their drivers. Meanwhile, unless a rapid shift away from fossil fuel use occurs worldwide, a doubling of carbon dioxide—and more—will be inevitable.

–RICHARD A. KERR

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What are human races, and how did they develop?
Anthropologists have long argued that race lacks biological reality. But our genetic makeup does vary with geographic origin and as such raises political and ethical as well as scientific questions.

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Why do some countries grow and others stagnate?
From Norway to Nigeria, living standards across countries vary enormously, and they're not becoming more equal.

What impact do large government deficits have on a country's interest rates and economic growth rate?
The United States could provide a test case.

LYLE CONRAD/CDC

Are political and economic freedom closely tied?
China may provide one answer.

Why has poverty increased and life expectancy declined in sub-Saharan Africa?
Almost all efforts to reduce poverty in sub-Saharan Africa have failed. Figuring out what will work is crucial to alleviating massive human suffering.

What Can Replace Cheap Oil—and When

The road from old to new energy sources can be bumpy, but the transitions have gone pretty smoothly in the past. After millennia of dependence on wood, society added coal and gravity-driven water to the energy mix. Industrialization took off. Oil arrived, and transportation by land and air soared, with hardly a worry about where the next log or lump of coal was coming from, or what the explosive growth in energy production might be doing to the world.

Times have changed. The price of oil has been climbing, and ice is melting around both poles as the mercury in the global thermometer rises. Whether the next big energy transition will be as smooth as past ones will depend in large part on three sets of questions: When will world oil production peak? How sensitive is Earth's climate to the carbon dioxide we are pouring into the atmosphere by burning fossil fuels? And will alternative energy sources be available at reasonable costs? The answers rest on science and technology, but how society responds will be firmly in the realm of politics.

There is little disagreement that the world will soon be running short of oil. The debate is over how soon. Global demand for oil has been rising at 1% or 2% each year, and we are now sucking almost 1000 barrels of oil from the ground every second. Pessimists—mostly former oil company geologists—expect oil production to peak very soon. They point to American geologist M. King Hubbert's successful 1956 prediction of the 1970 peak in U.S. production. Using the same method involving records of past production and discoveries, they predict a world oil peak by the end of the decade. Optimists—mostly resource economists—argue that oil production depends more on economics and politics than on how much happens to be in the ground. Technological innovation will intervene, and production will continue to rise, they say. Even so, midcentury is about as far as anyone is willing to push the peak. That's still "soon" considering that the United States, for one, will need to begin replacing oil's 40% contribution to its energy consumption by then. And as concerns about climate change intensify, the transition to nonfossil fuels could become even more urgent (see p. 100).

If oil supplies do peak soon or climate concerns prompt a major shift away from fossil fuels, plenty of alternative energy supplies are waiting in the wings. The sun bathes Earth's surface with 86,000 trillion watts, or terawatts, of energy at all times, about 6600 times the amount used by all humans on the planet each year. Wind, biomass, and nuclear power are also plentiful. And there is no shortage of opportunities for using energy more efficiently.

Of course, alternative energy sources have their issues. Nuclear fission supporters have never found a noncontroversial solution for disposing of long-lived radioactive wastes, and concerns over liability and capital costs are scaring utility companies off. Renewable energy sources are diffuse, making it difficult and expensive to corral enough power from them at cheap prices. So far, wind is leading the way with a global installed capacity of more than 40 billion watts, or gigawatts, providing electricity for about 4.5 cents per kilowatt hour.

That sounds good, but the scale of renewable energy is still very small when compared to fossil fuel use. In the United States, renewables account for just 6% of overall energy production. And, with global energy demand expected to grow from approximately 13 terawatts a year now to somewhere between 30 and 60 terawatts by the middle of this century, use of renewables will have to expand enormously to displace current sources and have a significant impact on the world's future energy needs.

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What needs to happen for that to take place? Using energy more efficiently is likely to be the sine qua non of energy planning—not least to buy time for efficiency improvements in alternative energy. The cost of solar electric power modules has already dropped two orders of magnitude over the last 30 years. And most experts figure the price needs to drop 100-fold again before solar energy systems will be widely adopted. Advances in nanotechnology may help by providing novel semiconductor systems to boost the efficiency of solar energy collectors and perhaps produce chemical fuels directly from sunlight, CO_2, and water.

But whether these will come in time to avoid an energy crunch depends in part on how high a priority we give energy research and development. And it will require a global political consensus on what the science is telling us.

–RICHARD A. KERR AND ROBERT F. SERVICE

The following six mathematics questions are drawn from a list of seven outstanding problems selected by the Clay Mathematics Institute. (The seventh problem is discussed on p. 96.) For more details, go to www.claymath.org/millennium.

Is there a simple test for determining whether an elliptic curve has an infinite number of rational solutions?
Equations of the form $y^2 = x^3 + ax + b$ are powerful mathematical tools. The Birch and Swinnerton-Dyer conjecture tells how to determine how many solutions they have in the realm of rational numbers—information that could solve a host of problems, if the conjecture is true.

continued >>

Can a Hodge cycle be written as a sum of algebraic cycles?
Two useful mathematical structures arose independently in geometry and in abstract algebra. The Hodge conjecture posits a surprising link between them, but the bridge remains to be built.

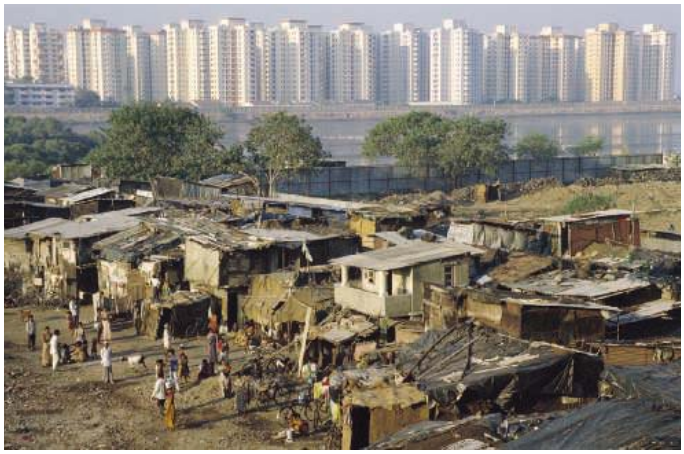
Will Malthus Continue to Be Wrong

In 1798, a 32-year-old curate at a small parish church in Albury, England, published a sobering pamphlet entitled *An Essay on the Principle of Population*. As a grim rebuttal of the utopian philosophers of his day, Thomas Malthus argued that human populations will always tend to grow and, eventually, they will always be checked—either by foresight, such as birth control, or as a result of famine, war, or disease. Those speculations have inspired many a dire warning from environmentalists.

Since Malthus's time, world population has risen sixfold to more than 6 billion. Yet happily, apocalyptic collapses have mostly been prevented by the advent of cheap energy, the rise of science and technology, and the green revolution. Most demographers predict that by 2100, global population will level off at about 10 billion.

The urgent question is whether current standards of living can be sustained while improving the plight of those in need. Consumption of resources—not just food but also water, fossil fuels, timber, and other essentials—has grown enormously in the developed world. In addition, humans have compounded the direct threats to those resources in many ways, including by changing climate (see p. 100), polluting land and water, and spreading invasive species.

How can humans live sustainably on the planet and do so in a way that manages to preserve some biodiversity? Tackling that question involves a broad range of research for natural and social scientists. It's abundantly clear, for example, that humans are degrading many ecosystems and hindering their ability to provide clean water and other "goods and services" (*Science*, 1 April, p. 41). But exactly how bad is the situation? Researchers need better information on the status and trends of wetlands, forests, and other areas. To set priorities, they'd also like a better understanding of what makes ecosystems more resistant or vulnerable and whether stressed ecosystems, such as marine fisheries, have a threshold at which they won't recover.



Out of balance. Sustaining a growing world population is threatened by inefficient consumption of resources—and by poverty.

Agronomists face the task of feeding 4 billion more mouths. Yields may be maxing out in the developed world, but much can still be done in the developing world, particularly sub-Saharan Africa, which desperately needs more nitrogen. Although agricultural biotechnology clearly has potential to boost yields and lessen the environmental impact of farming, it has its own risks, and winning over skeptics has proven difficult.

There's no shortage of work for social scientists either. Perverse subsidies that encourage overuse of resources—tax loopholes for luxury Hummers and other inefficient vehicles, for example—remain a chronic problem. A new area of activity is the attempt to place values on ecosystems' services, so that the price of clear-cut lumber, for instance, covers the loss of a forest's ability to provide clean water. Incorporating those "externalities" into pricing is a daunting challenge that demands much more knowledge of ecosystems. In addition, economic decisions often consider only net present value and discount the future value of resources—soil erosion, slash-and-burn agriculture, and the mining of groundwater for cities and farming are prime examples. All this complicates the process of transforming industries so that they provide jobs, goods, and services while damaging the environment less.

Researchers must also grapple with the changing demographics of housing and how it will impact human well-being: In the next 35 to 50 years, the number of people living in cities will double. Much of the growth will likely happen in the developing world in cities that currently have 30,000 to 3 million residents. Coping with that huge urban influx will require everything from energy-efficient ways to make concrete to simple ways to purify drinking water.

And in an age of global television and relentless advertising, what will happen to patterns of consumption? The world clearly can't support 10 billion people living like Americans do today. Whether science—both the natural and social sciences—and technology can crank up efficiency and solve the problems we've created is perhaps the most critical question the world faces. Mustering the political will to make hard choices is, however, likely to be an even bigger challenge. —ERIK STOKSTAD

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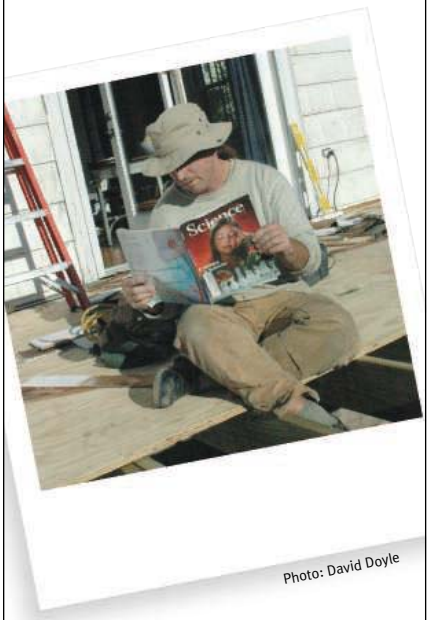
Will mathematicians unleash the power of the Navier-Stokes equations?
First written down in the 1840s, the equations hold the keys to understanding both smooth and turbulent flow. To harness them, though, theorists must find out exactly when they work and under what conditions they break down.

Does Poincaré's test identify spheres in four-dimensional space?
You can tie a string around a doughnut, but it will slide right off a sphere. The mathematical principle behind that observation can reliably spot every spherelike object in 3D space. Henri Poincaré conjectured that it should also work in the next dimension up, but no one has proved it yet.

Do mathematically interesting zero-value solutions of the Riemann zeta function all have the form a + bi?
Don't sweat the details. Since the mid-19th century, the "Riemann hypothesis" has been *the* monster catfish in mathematicians' pond. If true, it will give them a wealth of information about the distribution of prime numbers and other long-standing mysteries.

Does the Standard Model of particle physics rest on solid mathematical foundations?
For almost 50 years, the model has rested on "quantum Yang-Mills theory," which links the behavior of particles to structures found in geometry. The theory is breathtakingly elegant and useful—but no one has proved that it's sound.

Who's helping build the future of science?



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AAAS member Milton Trimitsis

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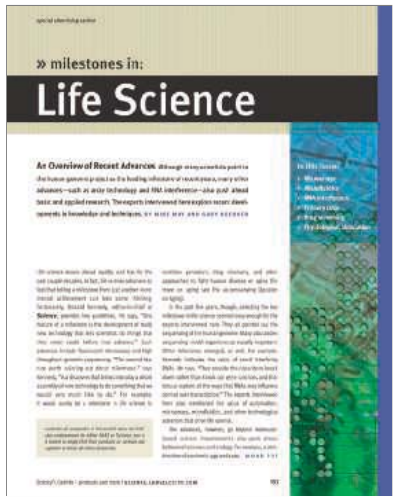

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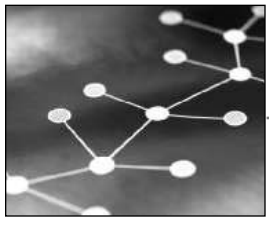

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Genome Analysis Reveals Pili in Group B *Streptococcus*

Peter Lauer,[1]* Cira D. Rinaudo,[1] Marco Soriani,[1] Immaculada Margarit,[1] Domenico Maione,[1] Roberto Rosini,[1] Anna Rita Taddei,[2] Marirosa Mora,[1] Rino Rappuoli,[1] Guido Grandi,[1] John L. Telford[1]†

Group B *Streptococcus* (GBS) is the major cause of neonatal sepsis in developed countries. Maternal opsonic antibodies to surface polysaccharide structures can cross the placenta and correlate with protection of the child (*1*). While screening the genomes of multiple GBS strains (*2*), we identified two surface exposed antigens, GBS80 (TIGR annotation SAG0645) and GBS104 (SAG0649), that mediated complement-dependent, opsonophagocytic killing of virulent GBS bacteria and conferred passive protection against GBS challenge in a mouse maternal immunization model (*3*). The genes coding for these two proteins are part of an operon containing five genes (Fig. 1A). GBS80, GBS104, and a third protein, GBS52 (SAG0646), contain the LPXTG motif found in surface proteins usually attached to the cell wall peptidoglycan. The other two genes (SAG0647 and SAG0648) code for sortase enzymes similar to those known to catalyze the covalent linkage of LPXTG motif proteins to the peptidoglycan (*4*).

Mouse antisera raised against recombinant GBS80 and GBS104 proteins stained the surface of intact GBS bacteria of strain COH1 (serotype III) and strain JM9130013 (serotype VIII) in flow cytometry (*5*). We separated total protein extracts of bacteria from these strains on reducing SDS–polyacrylamide gel electrophoresis (PAGE) and immunoblotted them with antisera specific for the GBS80 and GBS104 proteins (*6*). In addition to bands corresponding to the predicted molecular weights of the monomeric proteins, both antisera revealed a ladder of bands ranging from 150 kD to beyond the resolution of the 3 to 8% gradient gels used (Fig. 1B).

Immunogold electron microscopy of GBS80 (*6*) revealed pilus-like structures extending from the bacterial surface (Fig. 1C). In a strain carrying a plasmid that overexpressed GBS80 (*6*), antisera to GBS80 stained extremely long structures on the bacterial surface (Fig. 1D). Antisera specific for GBS104 also stained the pilus-like structures, but much less intensely (*5*). Neither immunogold labeling nor the high molecular weight ladder was observed with GBS80 antisera in a strain lacking the GBS80 gene; however, staining was recovered when we transformed this strain with a plasmid expressing GBS80 (fig. S1). Deletion of the genes coding for sortases 647 and 648 revealed that both are required for the correct assembly of the pilus (*5*). Thus, the high molecular weight covalent polymers detected by SDS-PAGE corresponded to pilus-like structures, and the length of the pili appeared to depend on the level of expression of GBS80.

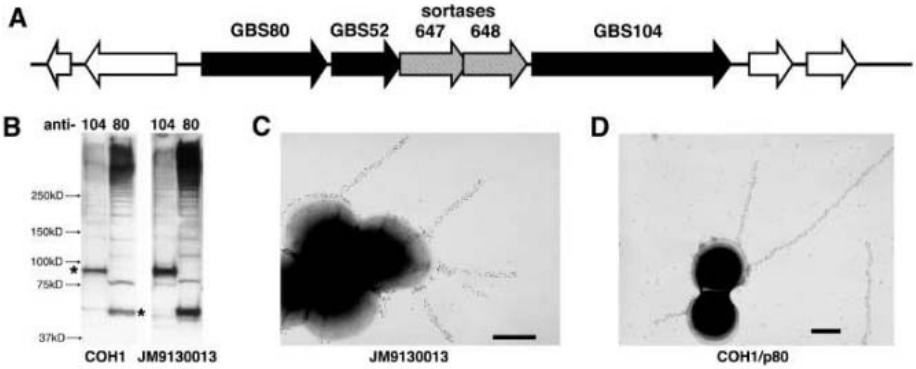

Fig. 1. (**A**) Schematic representation of the operon containing GBS80 and GBS104. (**B**) Immunoblots of total protein extracts of GBS bacteria with antisera specific for GBS80 and GBS104. The antisera used are given above the lanes, and the strains used are below the lanes. The positions of the monomeric forms of GBS80 (53 kD) and GBS104 (91 kD) are marked by asterisks. (**C**) Immunogold labeling and transmission electron microscopy of GBS80 in strain JM9130013, showing long pilus-like structures. (**D**) Immunogold staining of GBS80 in a strain of GBS (COH1) transformed with a plasmid (pGBS80) capable of overexpressing the protein. Scale bars, 500 nm.

Numerous pili or fimbriae are essential virulence factors and protective antigens in Gram-negative bacteria (e.g., *Neisseria meningitidis* and *N. gonorrhoeae*), where they are involved in adhesion of the bacteria to eukaryotic cells (*7*). In Gram-positive bacteria, pili have been described in *Corynbacterium diphtheriae*, where they are formed by covalent polymerization of pilin subunits catalyzed by particular sortase enzymes (*8*). Pilus-like structures have also been detected in some other Gram-positive bacteria (*9*); however, very little is known about their function, and they have not been described in any of the most important species of *Streptococcus* that are pathogenic to humans: GBS, Group A *Streptococcus*, and *Streptococcus pneumoniae*.

The presence in GBS of pilus-like structures composed of antigens that confer protection in a mouse model of maternal immunization suggests that pili may play an important role in the virulence of Gram-positive bacteria as well as in Gram-negative. These macromolecular structures, which are as long as the bacteria, may not have been detected by conventional approaches in Group B *Streptococcus* because they are not readily visible in electron microscopy of samples prepared by standard negative staining techniques. Genome surveys may therefore reveal other important features of pathogens hitherto missed by classical methodologies.

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Fig. S1
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Discovery of Pulsed OH Maser Emission Stimulated by a Pulsar

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Stimulated emission of radiation has not been directly observed in astrophysical situations up to this time. Here we demonstrate that photons from pulsar B1641−45 stimulate pulses of excess 1720-megahertz line emission in an interstellar hydroxyl (OH) cloud. As this stimulated emission is driven by the pulsar, it varies on a few-millisecond time scale, which is orders of magnitude shorter than the quickest OH maser variations previously detected. Our 1612-megahertz spectra are inverted copies of the 1720-megahertz spectra. This "conjugate line" phenomenon enables us to constrain the properties of the interstellar OH line–producing gas. We also show that pulsar signals undergo significantly deeper OH absorption than do other background sources, which confirms earlier tentative findings that OH clouds are clumpier on small scales than are neutral hydrogen clouds.

Pulsars are outstanding tools for the study of the interstellar medium (ISM). Their pulsed signals undergo a variety of modifications as they propagate through the ISM, revealing extensive information about the global distribution and physical properties of the intervening material. The tiny sizes of pulsars ensure that their signals probe very small transverse scales in the ISM (*1*, *2*). Another virtue of pulsar ISM measurements is that the pulsars cycle rapidly on and off, so that observations may be made contemporaneously in both the presence and absence of the pulse, and the properties of the medium can be precisely compared in these two states. For example, the comparison of neutral hydrogen (HI) spectra acquired during and between pulses leads to pulsar absorption spectra that can be used for determinations of kinematic distance and interstellar electron density (*3*–*5*). Recently, Stanimirovic *et al.* (*6*) extended the pulsar spectral technique to the OH molecule with the first successful OH absorption measurements toward pulsar PSR B1849+00. We expanded OH spectral measurements to 18 additional pulsars, chosen because they are relatively bright and lie in the inner Galaxy near the galactic plane. One of them, B1641−45 = PSR J1644−4559, not only exhibits intervening OH absorption at 1612, 1665, and 1667 MHz, but also shows interstellar stimulated emission at 1720 MHz.

The widths and strengths of spectral lines from some interstellar molecules provide abundant indirect evidence for stimulated emission processes; soon after the discovery of interstellar OH in the 1960s, it was recognized that maser processes must be involved (*7*–*9*). Beam-switched OH measurements toward the radio galaxy 3C123 have also demonstrated that a background source can stimulate emission in interstellar OH (*10*). The pulsed 1720-MHz maser detection reported here represents direct astronomical observation of the process of the radiative stimulation of emission. The broadband pulsar spectrum exhibits excess line emission at 1720 MHz as the pulsar's photons stimulate the creation of additional photons in an intervening OH cloud. This excess emission switches on and off with the pulsar, clearly demonstrating its stimulated nature. We analyzed these pulsar absorption and stimulated emission observations, and compare them here with similar measurements in nonpulsar observations to study the physical properties of the intervening medium.

A pulsar-binning spectrometer was used to ultimately create two separate spectra: a "pulsar" spectrum and a "pulsar-off" spectrum (*11*). The pulsar spectrum represents the signal of the pulsar alone, as absorbed or amplified by intervening OH. In contrast, the pulsar-off spectrum is sensitive to OH emission or absorption occurring anywhere within the telescope beam. The main OH lines at 1665 and 1667 MHz were simultaneously observed in the 4-MHz bandpass with several-hour integrations for each of the 18 pulsars in our sample. (See Table 1 for total integration times and for 1σ optical depth uncertainties in the Hanning smoothed pulsar spectra.) After our success in detecting absorption in the 1665- and 1667-MHz pulsar spectra of PSR B1641−45, we also measured the satellite (1612- and 1720-MHz) lines toward the pulsar.

In order to calculate the optical depth τ of absorption or stimulated emission, we considered the defining equation for optical depth, $I/I_o = e^{-\tau}$, where I_o is the original intensity measured offline and I is the intensity after traversal through optical depth τ of material. The differencing procedure leading to the pulsar spectrum automatically eliminates all nonpulsar signals and yields the spectrum in units of $(I/I_o)|_{PSR}$, so it is straightforward to calibrate the pulsar spectrum in terms of τ. It is not so simple to determine optical depths in the pulsar-off spectra for several reasons. First, it is not clear whether the background emission and foreground absorption/amplification regions subtend the same solid angle. It is probably reasonable to assume in our case that the background fills the telescope beam, because its predominant source is the smoothly distributed galactic nonthermal emission, but the size of the foreground clouds is unknown from our measurements. Consequently, in the absence of better information, we will assume that both foreground and background fill the beam, so that $I/I_o = T/T_o$, where T is the original brightness temperature (measured offline) and T_o is the brightness temperature after traversal through the materi-

Table 1. Integration times and pulsar spectrum noise fluctuations.

PSR J	PSR B	Freq (MHz)	t_{tot} (hour)	σ_τ* (pulsar spectrum)
0742-2822	0740-28	1665/7	3	0.1
0835-4510	0833-45	1665/7	2	0.1
0837-4135	0835-41	1665/7	4	0.07
0908-4913	0906-49	1665/7	4	0.1
1056-6258	1054-62	1665/7	2	0.1
1057-5226	1055-52	1665/7	1	0.1
1157-6224	1154-62	1665/7	2	0.3
1243-6423	1240-64	1665/7	2	0.1
1326-5859	1323-58	1665/7	2	0.1
1327-6222	1323-62	1665/7	2	0.1
1600-5044	1557-50	1665/7	7	0.1
1605-5257	1601-52	1665/7	1	0.2
1644-4559	1641-45	1665/7	5	0.01
1644-4559	1641-45	1720	5	0.01
1644-4559	1641-45	1612	4	0.01
1745-3040	1742-30	1665/7	5	0.2
1752-2806	1749-28	1665/7	4	0.05
1803-2137	1800-21	1665/7	5	0.3
1825-0935	1822-09	1665/7	1	0.3
1829-1751	1826-17	1665/7	2	0.3

*The quantity σ_τ (pulsar spectrum) is the optical depth standard deviation in the Hanning smoothed pulsar spectrum, which includes the effects of radiometer and sky noise, and in some cases, interstellar scintillation.

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al (see below). However, another difficulty is that the observed continuum is emitted throughout the line of sight across the Galaxy, whereas the definition of τ requires that T_o be only that portion emitted beyond the cloud contributing to optical depth. In order to estimate the fraction of the continuum $f(d)$ contributing to T_o as a function of distance d, we synthesized a model of the continuum emission $\epsilon(s)$ as a function of distance s along the line of sight, consisting of galactic synchrotron (*12*), ionized hydrogen regions, and the 2.7-K cosmic background radiation. We then integrated this model along the appropriate (background) part of the line of sight and normalized the result by the total emission along the line of sight

$$f(d) = \frac{\epsilon_{\text{background}}}{\epsilon_{\text{tot}}} = \frac{\int_d^\infty \epsilon(s)ds}{\int_0^\infty \epsilon(s)ds} \qquad (1)$$

Then $T_o(d) = T_{bo} f(d)$, where T_{bo} is the brightness temperature offline, determined as described in (*11*). Combining all the above results for pulsar-off spectra, we find that $\tau = -\ln(I/I_o) = -[T/T_o(d)] = -\ln[T/f(d)T_{bo}(d)]$. Distance was then mapped to radial velocity of the spectra with a galactic rotation model (*13*).

Figure 1 displays the 1720-MHz spectra toward PSR B1641−45, which directly demonstrate the process of stimulated emission. The pulsar-off spectrum (Fig. 1A, bottom), which was acquired in the interval between pulses, shows both emission and absorption against other background source(s) lying within the 13–arc min telescope beam. The pulsar-on spectrum (Fig. 1A, top) appears to zeroth order to be merely a copy of the pulsar-off spectrum, shifted upward by a constant equal to the broadband pulsar signal strength. However, when these two spectra are carefully differenced (*11*) to create the pulsar spectrum (Fig. 1B), it is clear that there is excess signal at velocity (v) ~ −45 km/s, where the broadband pulsar signal has been amplified by stimulated emission. It has long been thought that stimulated emission plays an important role in astrophysical OH line radiation. For example, line temperatures are frequently far in excess of those inferred from (assumed thermal) line widths (*7–9*). However, our measurements directly demonstrate the stimulated amplification of a signal propagating through the ISM, in that the amplification is directly observable as the pulsar cycles on and off during its 455-ms rotational period. As the stimulated emission switches on and off synchronously with the pulsar pulse, our 14-ms time resolution [1/32nd of the pulse period (*14*)] places a very short upper limit on its duration. Because the shortest intrinsic fluctuation time scale previously reported was ~1000 s (*15*), these variations are by far the quickest observed in any interstellar maser.

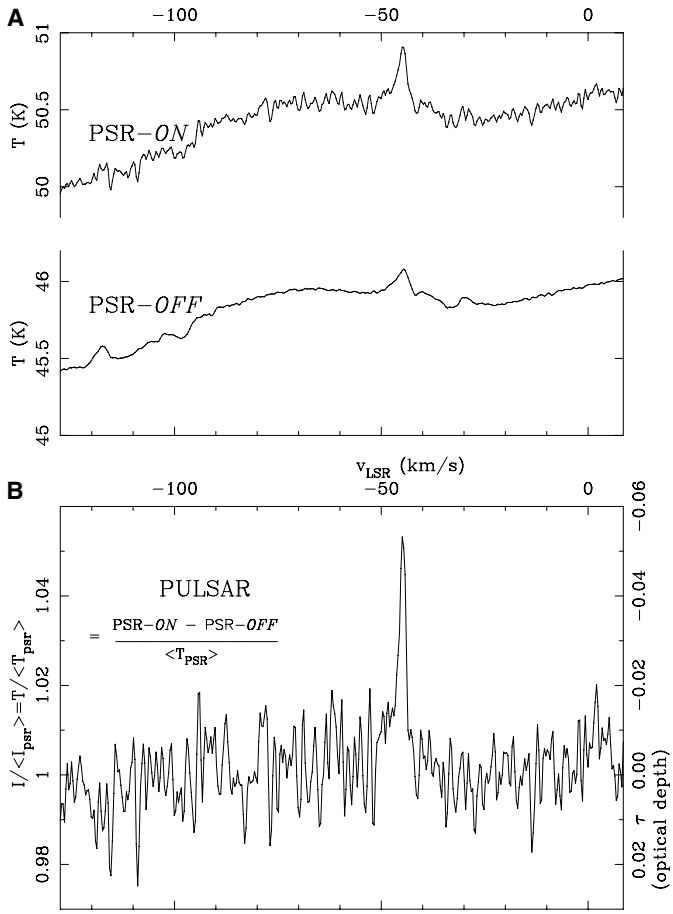

Fig. 1. Stimulated amplification of the PSR B1641−45 signal in an interstellar OH cloud at 1720 MHz. **(A)** The pulsar-on (top) spectrum, acquired during the pulsar pulse, and (bottom) the pulsar-off spectrum, gathered in the interval between pulses. The two spectra exhibit both emission and absorption against other (nonpulsar) background source(s) lying within the 13–arc min telescope beam, whereas the pulsar-on spectrum additionally contains the pulsar signal. **(B)** The pulsar spectrum, the difference of pulsar-on and pulsar-off spectra, illustrating the pulsar signal alone as absorbed (or in this case, amplified) by intervening OH. The spike in this spectrum at v_{LSR} ~ −45 km/s results from excess emission in an OH cloud, stimulated by pulsar photons.

Two conditions must be satisfied in order for stimulated emission to occur. First, the populations at the relevant levels must be inverted or pumped by some process. Second, appropriate photons should be available to stimulate the emission from the upper overpopulated level. In our case, the level inversion is accomplished locally in the OH cloud by a low-energy radiative or collisional process, whereas the stimulating photons are provided by the pulsar.

The pulsed maser line optical depth τ ~ −0.05, which implies that approximately five excess line photons are stimulated in the cloud for every hundred passing through it. Because the maser is unsaturated with a gain of only 1.05, we expect that the full width at half maximum (FWHM) of the line should be very similar to the expected thermal line width, which is about 0.5 to 0.7 km/s for gas with kinetic temperature of 100 to 200 K. For example, a typical FWHM of 0.5 km/s was found in a large survey of 1720-MHz masers in star-forming regions (*16*). The FWHM we measure is about 2 km/s, which is slightly wider and suggests that we are most likely seeing a blend of several maser spots along the line of sight.

It has been suggested (*17*) that extraterrestrial civilizations could use interstellar masers to amplify their radio transmissions. We have demonstrated here that such a process could sustain modulation down to millisecond time scales, but of course the gain of this particular maser is too small to provide significant amplification of an extraterrestrial intelligent signal.

PSR B1641−45 = J1644−4559 lies in a well-studied (*18–22*) region of the inner Galaxy near the galactic plane, at galactic longitude l and latitude b (l, b) = (339.2°, −0.2°). We were able to construct a schematic map of the ISM along the line of sight by combining our observations with earlier ones (Fig. 2).

On the basis of kinematic analysis of HI absorption spectra (*23*), the pulsar is placed 4.6 kpc along the line of sight. Two ionized hydrogen (HII) regions lie in this direction, with galactic coordinates and recombination line velocities with respect to the local standard of rest (LSR), v_{LSR} (*19*, *20*) of (l, b, v_{LSR}) = (339.1°, −0.2°, −120 km/s) and (339.1°, −0.4°, −37 km/s). With the rotation curve of (*13*), our kinematic analysis of the recombination line measurements places G339.1-0.2 beyond the pulsar at a geocentric distance d ~ 6.7 kpc and places G339.1-0.4 closer than the pulsar at d ~ 3.3 kpc.

Figure 3 displays spectra at frequencies of the four ground–rotational state, 18-cm

OH lines toward PSR B1641−45. There is a strong spectral line at $v_{LSR} \sim -45$ km/s in all of our OH spectra, both pulsar-off and pulsar spectra. The line is in absorption at 1612, 1667, and 1665 MHz and in emission at 1720 MHz (the latter being the pulsed maser emission discussed above). Because it is visible in the pulsar spectra (Fig. 3, right column), this line must arise between the pulsar and the observer. It probably originates in OH gas associated with or near G339.1-0.4, because the velocities are similar. However, pervasive extended regions of 1720-MHz emission have been found in the inner galactic plane (*24*, *25*), including a >1°-long filament crossing near the pulsar line of sight with $v_{LSR} \sim -40$ km/s. Because our 1720-MHz pulsar-off spectral line at −45 km/s has strength and width similar to those of the extended OH gas (although the velocities are somewhat discrepant), it is possible that it originates from this extended OH region rather than from the HII region G339.1-0.4.

The −45-km/s emission and absorption features show evidence for departures from local thermodynamic equilibrium (LTE). The pulsar-off spectra typically have FWHM of 2 to 3 km/s, which is a few times wider than what is expected for thermally broadened line profiles at a typical kinetic temperature of about 100 K. The peak optical depths are very similar at 1667 and 1665 MHz ($\tau \sim 0.03$), whereas in the LTE case, their ratio would be 9/5, respectively. In addition, the 1612 MHz lines are inverted with respect to 1720 MHz.

We see another OH line at $v_{LSR} \sim -30$ km/s in most of the eight spectra toward PSR B1641−45 shown in Fig. 3, including the 1665- and 1667-MHz pulsar spectra. This line must therefore also originate in gas nearer to us than the pulsar, probably associated with or near G339.1-0.4. The lines are seen in absorption in pulsar spectra and primarily in emission in pulsar-off spectra.

Finally, all pulsar-off spectra exhibit line(s) at $v_{LSR} \sim -100$ to −120 km/s, which are not seen in the pulsar spectra. Consequently, they must originate in gas beyond the pulsar, probably associated with or near G339.1-0.2.

Each 1720-MHz spectrum (Fig. 3, top row) is an inverted copy of the 1612-MHz spectrum (Fig. 3, second row). This phenomenon, called conjugate line behavior, occurs because the initial states of both transitions are overpopulated by an identical process (*8*, *26–28*). For our predominantly observed conjugate state, which has 1720-MHz stimulated emission and 1612-MHz stimulated absorption, the process begins in a region with $T \sim 100$ K and OH number density $n_{OH} \sim 10^5$ cm^{-3} with the collisional excitation of the molecule to a higher rotational state at energy $E = 1.66 \times 10^{-14}$ ergs above ground level, after which it can radiatively decay with equal probability (if the transition is optically thick) to overpopulate either the upper level of the 1720-MHz transition or the lower level of the 1612-MHz transition. The 1612-MHz stimulated emission and conjugate 1720-MHz stimulated absorption, which we observed more rarely, result from a similar process that overpopulates the opposite 18-cm levels via an intermediate excited rotational level at $E = 2.5 \times 10^{-14}$ ergs above the ground level.

The predominant conjugate configuration becomes optically thick to far-infrared photons for OH column densities N_{OH} per velocity interval $N_{OH}/\Delta v > 10^{14}$ s km^{-1} cm^{-2}, whereas the inverse configuration becomes optically thick and then dominates at $N_{OH}/\Delta v > 10^{15}$ s km^{-1} cm^{-2}. Hence our predominant conjugate configuration (including the 1720-MHz pulsar-stimulated emission and the pulsar-off emission at $v \sim -120$ km/s) originates in clouds with specific column densities between these two limits, whereas the rarer opposite configuration (e.g., pulsar-off 1720-MHz absorption at $v \sim -100$ km/s) originates in a column whose density is above the upper limit. Then the occasionally observed adjacent emission and absorption features that are conjugate at the two frequencies (e.g., the pulsar-off spectra at $v \sim -32$ km/s) suggest a density gradient in the cloud (*28*), with specific column densities crossing 10^{15} s km^{-1} cm^{-2} at the transition.

We compared the lines observed in the pulsar and pulsar-off spectra, because all the lines were acquired at the same time, with the telescope pointing in exactly the same direction. To facilitate the comparison, optical depth scales on the right side of all eight spectra in Fig. 3 are identical. The lines where $v \sim -45$ km/s exhibit markedly stronger ($|\tau| \sim$ 2 to 3 times larger) absorption and stimulated emission in pulsar spectra (Fig. 3, right column) than those in the corresponding pulsar-off spectra (Fig. 3, left column). The discrepancy at $v \sim -32$ km/s is even stronger; absorption in pulsar spectra at 1665 and 1667 MHz is absent or replaced by weak emission in pulsar-off spectra (*29*). The only other successful pulsar OH absorption experiment (*6*) also found stronger absorption in the pulsar spectra than in the pulsar-off spectra.

The widths of the lines are narrower in our pulsar spectra than in pulsar-off spectra at 1720 and 1612 MHz, but similar at 1667 and 1665 MHz. The earlier results (*6*) exhibited narrower lines in the pulsar spectra than in the pulsar-off spectra at 1667 and 1665 MHz.

Our observations strengthen the earlier interpretation that the needle-thin interstellar column sampled by the pulsar signal interacts with a substantially different sample of the medium than does the pulsar-off column, which represents the average of all interactions across the 13–arc min telescope beam. Presumably the pulsar signal is encountering small and dense OH cloudlets, whose properties are diluted in the beam-averaged pulsar-off spectrum. This behavior differs markedly from HI, where the statistics show no dependence on the angular cross-sections of absorbing columns across a tremendous range of

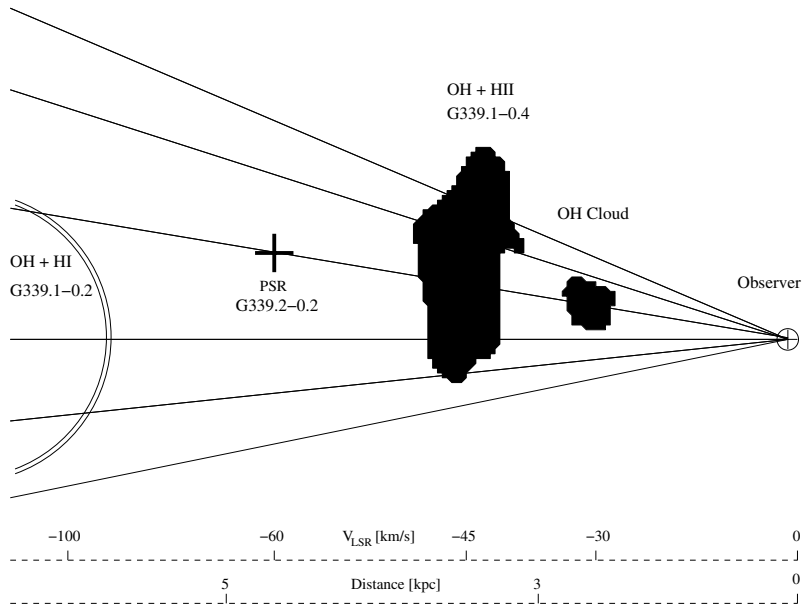

Fig. 2. A schematic model of the ISM toward PSR B1641−45. The ionized region velocities are from (*19*, *20*), the OH velocities are from our current work, and the limiting pulsar HI absorption velocities are from (*23*). The kinematic velocity-to-distance conversion uses the rotation curve of (*13*). The lines represent various lines of sight within the 13–arc min telescope beam. The vertical scale has been enlarged for clarity.

solid angles (*30*, *31*). Molecular gas is known to be more clumped than neutral gas, at least on larger scales.

If the difference in OH optical depths indeed results from clumping, we would expect that other pulsar lines of sight would pierce regions devoid of cloudlets and show shallower optical depths than pulsar-off measurements. One might ask why there are no such complementary results. Unfortunately, an observational selection effect prevents success in such observations, because we do not have sufficient sensitivity on any other pulsar lines of sight (Table 1) to test this hypothesis.

An earlier paper (*32*) reported broad ($\Delta v >$ 10 km/s) and deep ($\tau > 0.5$) OH absorption at 1667 MHz in the spectrum of PSR B1749−28. We have adequate sensitivity to detect such a line (Table 1), but we do not confirm the result. All other OH lines detected in pulsar spectra are much narrower than the previously claimed detection in PSR B1749−28.

We have searched for OH absorption and stimulated emission in the spectrum of 18 pulsars. One pulsar, B1641−45, exhibits absorption or stimulated emission in pulsar spectra at each of the four 18-cm line frequencies. No absorption or stimulated emission was detected in the others, including one in which OH absorption had previously been reported (B1749−28). A variety of results are drawn from the B1641−45 spectra. The pulsed maser line, with $\tau \sim -0.05$, represents the first direct detection of interstellar stimulated emission. The OH and HII concentrations are mapped along the line of sight to the pulsar, and they are found to be associated kinematically and probably spatially. Analysis of the lines provides insight into the OH density, temperature, and excitation. Finally, the relative depths of lines in pulsar spectra and pulsar-off spectra suggest that the OH gas is highly clumped.

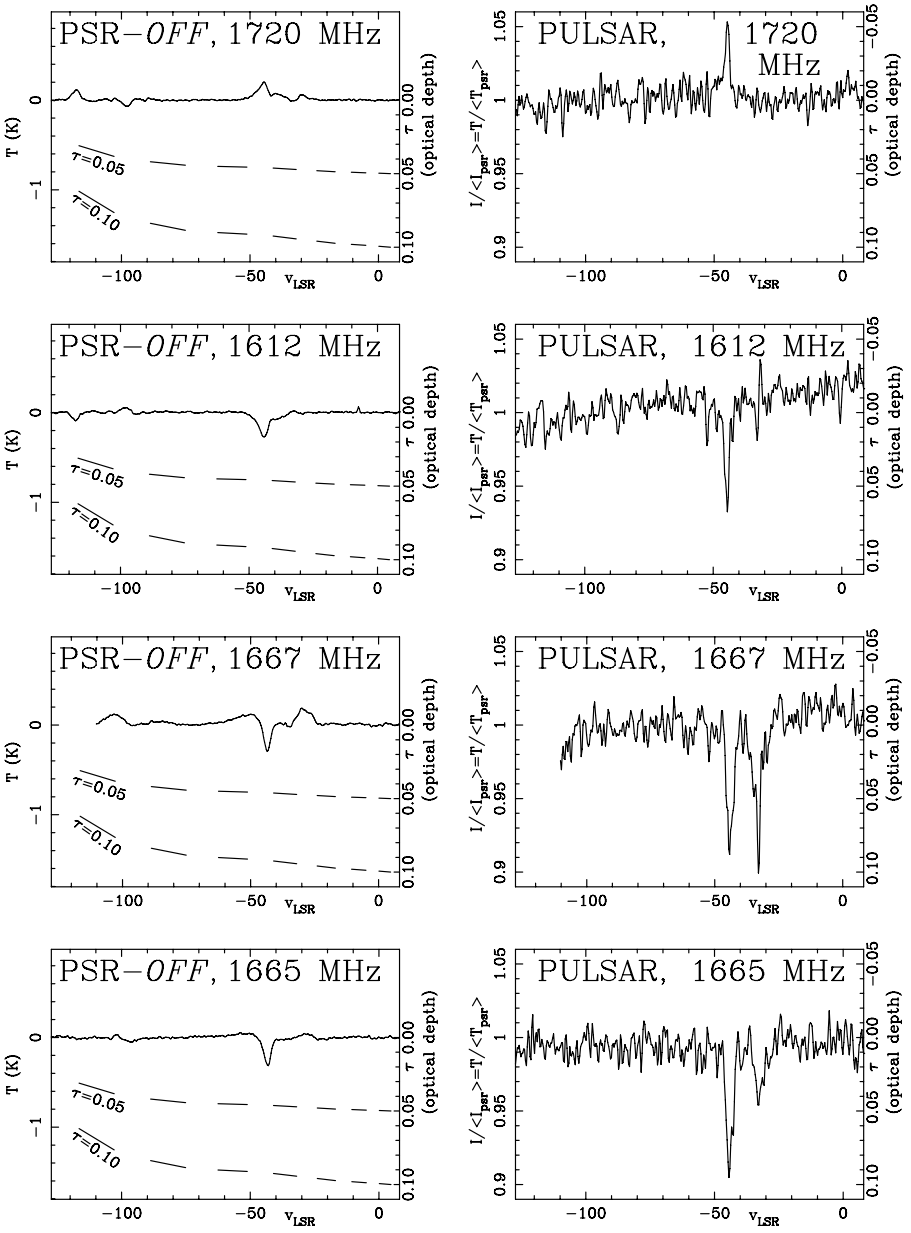

Fig. 3. Spectra of the four 18-cm ground-state rotational transitions of OH toward PSR B1641−45. The left column displays the four pulsar-off spectra, which are sensitive to all emission and absorption in the 13–arc min telescope beam when the pulsar is switched off. The right column shows the four pulsar spectra, which exhibit the absorption or stimulated emission of the pulsar signal alone. The righthand ordinate on each panel is optical depth τ; all eight spectra are plotted with the same optical depth scale. All pulsar-off spectral features are significantly shallower (i.e., have smaller optical depths) than their analogs in the pulsar spectra. In the pulsar-off spectra, the sloping lines of constant optical depth result from changes in the ratio of background to total continuum along the line of sight (see discussion accompanying Eq. 1). Low-order sinusoids were fitted to and removed from the pulsar-off baselines in order to flatten them.

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A High-Pressure Structure in Curium Linked to Magnetism

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Curium lies at the center of the actinide series and has a half-filled shell with seven 5*f* electrons spatially residing inside its radon core. As a function of pressure, curium exhibits five different crystallographic phases up to 100 gigapascals, of which all but one are also found in the preceding element, americium. We describe here a structure in curium, Cm III, with monoclinic symmetry, space group *C2/c*, found at intermediate pressures (between 37 and 56 gigapascals). Ab initio electronic structure calculations agree with the observed sequence of structures and establish that it is the spin polarization of curium's 5*f* electrons that stabilizes Cm III. The results reveal that curium is one of a few elements that has a lattice structure stabilized by magnetism.

The contribution of various factors in the electronic structure of a material to the bonding in its solid phase is at the heart of materials science and is a subject of extensive experimental and theoretical interest. It is well known that, when approaching the center of the actinide (5*f*) series of elements, a marked change occurs in the elemental volumes. The atomic volume of americium (Am) is almost 50% larger than that for the preceding element plutonium (Pu) (Fig. 1). The lighter actinides (Pa to Pu) have smaller atomic volumes and itinerant 5*f* states that participate in the (metallic) bonding and thus contribute to the cohesive properties of the solid. However, the 5*f* states are also capable of spin-polarization and hence magnetism. When the 5*f* bands are broad, as in the itinerant metals (Pa to Pu), there is an absence of magnetic correlations (*1*, *2*). However, for heavier actinide elements (Am and beyond), there is no 5*f* bonding, and magnetic correlations give rise to local moments, as found in the analogous 4*f* elements. Of particular interest with these heavier actinides is whether applied pressure can bring about the delocalization (a change of character from localized to itinerant) of their 5*f* electrons, and, if so, what are the consequent crystallographic, electronic, and magnetic structures?

In the periodic table, iron and cobalt are unique in the sense that the magnetic interactions between *d* electron states determine their crystal structures (*3*–*5*). Given that the magnetic correlations are between *f* electron states in the actinides, we may ask whether such magnetic interactions can influence the sequence of crystal structures.

There are fundamental differences in the pressure-volume relationships of the light (*6*, *7*) and heavy actinide metals (Fig. 1). Under compression, the relative volume changes with pressure for α-uranium (the room-temperature–stable form of uranium metal) (*7*) are clearly different from those for either Am or curium (Cm). We have investigated in detail the case of Am (*8*, *9*), where four crystal structures are found to exist between ambient pressure and 100 GPa. The delocalization of the 5*f* electrons of Am by pressure occurs in two stages, with the progressive formation of two lower symmetry structures, a face-centered orthorhombic Am III and a primitive orthorhombic structure, Am IV; the transition to each is accompanied by an abrupt decrease in the relative atomic volume. The formation of the Am IV structure (space group, *Pnma*), which was subsequently confirmed by theory (*10*), is now recognized as an important high-pressure structure for *f* electron metals.

In Cm, the $5f^7$ half-filled orbital provides a stabilizing effect. Consequently, forcing its 5*f* electrons to participate in its bonding requires higher pressures than in the case of Am. At ambient pressure, only the 6*d* 7*s* states of these elements are involved in their metallic bonding (*2*). With the application of pressure, the double hexagonal close packed (dhcp) form of Cm ($P6_3/mmc$, Cm I) converts to a face-centered cubic (fcc) structure (*Fm3m*, Cm II) at 17(2) GPa. This transformation requires little energy and reflects an increase in the *d* character of the bonding. There is a smooth transition between the Cm I and Cm II phases (Fig. 1), indicating that each phase has a comparable bulk modulus. This same transition occurs in Am, but at a lower pressure (6 GPa) (*8*, *9*).

Previous work (*11*, *12*) has identified the initial dhcp-fcc transition and also reported a phase transition above 40 GPa, but was unable to determine the correct structure. Given the pressure behavior of Am, one could have anticipated finding a structure similar to the Am III structure (*Fddd*) after the Cm II phase. However, our synchrotron radiation data show unambiguously that the Cm III phase is not *Fddd* as found for Am III. Before looking in detail at this Cm III phase, we will discuss the higher pressure phases of Cm.

Increasing the pressure above 56(4) GPa results in a third phase transition (Cm III to Cm IV), and this phase can indeed be identified with the *Fddd* structure as found for Am III. A smooth transition is observed between the Cm III and Cm IV phases. Above 95(5) GPa, the fourth phase transition (Cm IV to Cm V) is observed and yields a *Pnma* phase, which was previously identified for the Am IV structure. The *Fddd* to *Pnma* (Cm IV to Cm V) transition is accompanied by an ~11.7% volume collapse, whereas at the Cm II to Cm III transition, the collapse is ~4.5%. These abrupt volume changes signify the stepwise delocalization of the 5*f* electrons and their subsequent participation in the metallic bonding. In Am, the total collapse of ~9% for two transitions is smaller than that for Cm, but in both elements the collapses occur in two stages. The appearance of the *Fddd* and *Pnma* forms for Am (*8*, *9*) and Cm at higher pressures is a clear indication that the 5*f* delocalization process favors these structures.

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The puzzle, however, remains the formation of the Cm III phase. This phase, starting at ~37 GPa and extending to 56 GPa, has a monoclinic structure with the space group *C2/c*. A Rietveld fit of the Cm III data for this phase is shown in Fig. 2 at 45 GPa. This structure has not previously been reported for any element with f electrons.

An illustration of the different structures observed in Cm is given in Fig. 3. In comparison to the Am III or Cm IV (*Fddd*) structures, the Cm III (*C2/c*) structure is composed of slightly distorted (rectangularly distorted) close-packed hexagonal planes, but in contrast to the (*Fddd*) structure, it has a stacking arrangement that reduces the symmetry to monoclinic.

The isothermal bulk modulus (B_0) and its pressure derivative (B'_0) for Cm were determined from experimental data for the Cm I and Cm II low-pressure phases (localized *f* electrons) by fitting the experimental data to the Birch-Murnaghan (*13*) and Vinet (*14*) equations of state. Values of 36.5(3) GPa for B_0 and 4.6(2) for B'_0 were obtained with both equations. The inset of Fig. 1 shows the atomic volumes of the actinide metals at ambient pressure plotted together with their bulk moduli (*6–9*, *15–17*).

In an attempt to understand the stability of the unusual Cm III structure with its lower *C2/c* symmetry, we performed calculations using the full potential linear muffin-tin orbital (FPLMTO) method (*18–20*), in which basis functions, electron densities, and potentials are calculated without geometrical approximations. These quantities were expanded in spherical waves (with a cut-off maximum orbital angular momentum of 6) inside nonoverlapping spheres surrounding the atomic sites (muffin-tin spheres) and in a Fourier series in the interstitial region between the spheres. Total energy calculations were performed with two magnetic configurations, ferromagnetic (FM) and antiferromagnetic (AFM), as a function of volume. The calculations show that the AFM configuration is always lower in energy compared to the FM configuration for all structures. For example, for the Cm III structure, the difference in energy between the AFM and FM configurations at a volume of 16 Å^3 per atom (where Cm III is the stable phase) is around 30 millirydberg (mRy) per atom in favor of the AFM configuration. Total energy differences for different magnetic configurations over a wide volume range are shown in fig. S2. Furthermore, the calculations also show that the correct structural sequence can only be obtained if we treat all the structures in the AFM configuration.

Calculated total energy differences between the various structures using Cm II as the reference structure are shown in Fig. 4. The Cm III phase is theoretically stable between 17 and 15 Å^3, whereas experimentally it is found between 19.6 and 17.2 Å^3. In all cases, the theoretically derived critical volumes are smaller than those observed experimentally, but this is a general problem probably associated with the simulation of the core states. However, the relative sequence of phase transitions is reproduced. More importantly, the Cm III structure without magnetic correlations is not the favored crystal structure. By comparing enthalpies, we calculated the transition pressures for Cm III and found good agreement with those determined experimentally (table S1).

The calculated magnetic moment for the Cm I phase in the AFM state starts at almost 7 Bohr magnetons (μ_B), as expected for the half-filled shell and full spin polarization, and in agreement with experiments at ambient pressure (*21*). Theoretically, as the volume is decreased, the moment decreases gradually and disappears at the Cm IV to Cm V phase transition, when the 5*f* electrons are completely delocalized. In the Cm III phase, the calculated AFM moment decreases from 5 to 4 μ_B as the pressure is increased. Experiments giving the magnetic moment of Cm as a function of pressure have not been reported.

The experiments presented here on Cm, a pivotal element at the center of the 5*f* actinide series, have shown that it exhibits a complex sequence of phase transitions up to 100 GPa (1 Mbar). As expected, given the immediate proximity between Am and Cm in the periodic table and that, under high pressures, both will reach states with fully delocalized (itinerant) 5*f* behavior, the sequences of phase transitions as a function of pressure in Am (*8*, *9*) and Cm are similar. However, Cm exhibits an additional intermediate phase, Cm III, a monoclinic *C2/c* structure, that occurs before the 5*f* states become

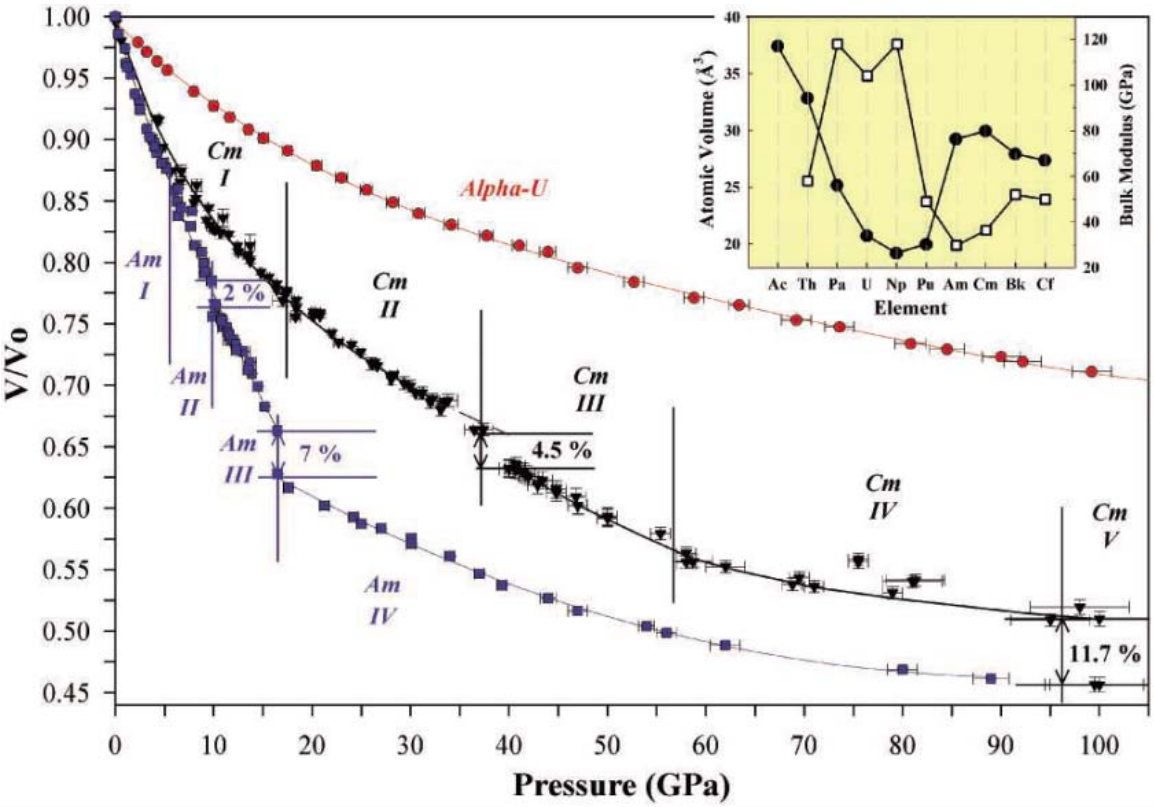

Fig. 1. Relative volume (V/V_0) as a function of pressure for α-uranium (*7*), Am (*8*, *9*) and Cm (this work). Vertical lines separate the pressure ranges for each Am and Cm (crystallographic) phase. Percentage values indicate the collapses in atomic volume. **(Inset)** Ambient pressure atomic volumes (solid circles, left-hand side) and bulk moduli (open squares, right-hand side) across the actinide series.

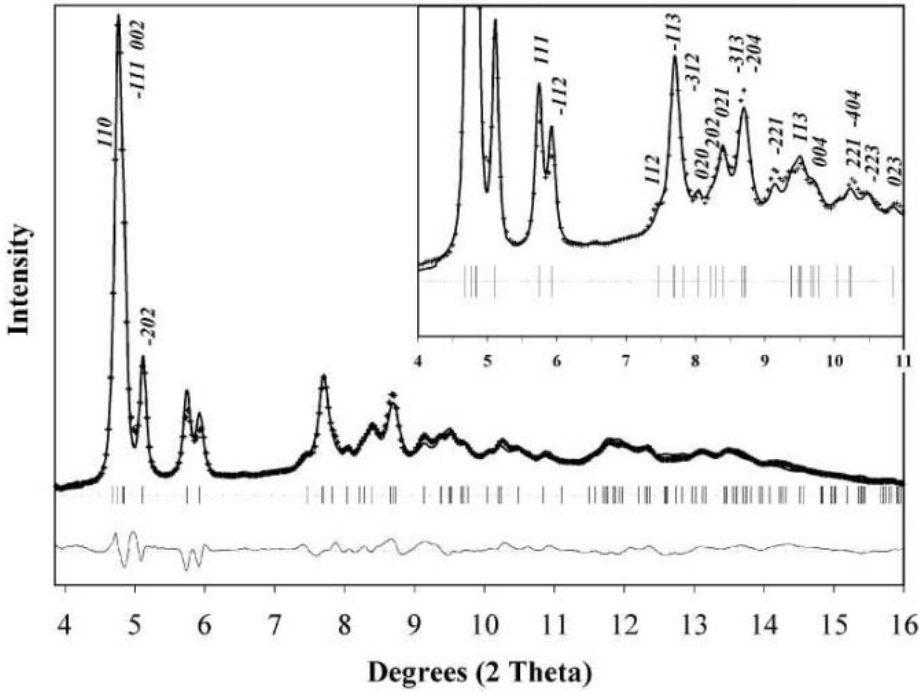

Fig. 2. Rietveld refinement of Cm III at 45 GPa, showing the observed (crosses) and calculated (line) diffraction patterns, reflection tick marks (vertical lines), principal Miller indices, and difference profile (lower line). The inset shows a detailed view of the strongest diffraction lines of the pattern, labeled by their Miller indices. Monoclinic space group *C2/c*, Cm on 4e sites ($x = 0$, $y = 0.1753$, $z = \frac{1}{4}$) has characteristics $a = 5.346(1)$ Å, $b = 2.886(1)$ Å, $c = 5.328(1)$ Å, $\beta = 116.2(2)°$, and Bragg-R Factor = 5.6%.

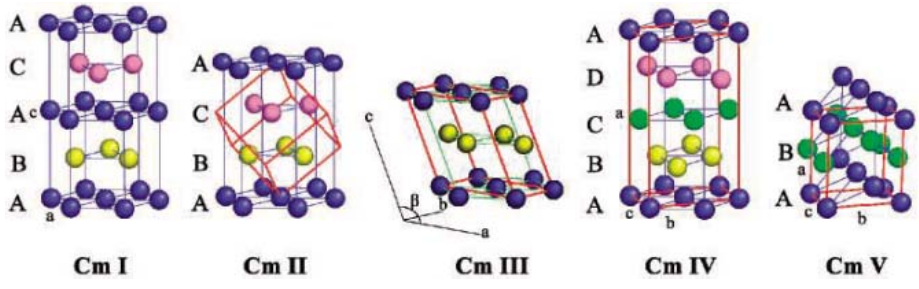

Fig. 3. Structural models of the Cm I to Cm V phases permit the visualization of transformation processes under pressure. The structures can be viewed as being composed of close-packed hexagonal planes (Cm I and Cm II) or distorted close-packed hexagonal planes (Cm III, Cm IV, and Cm V), with a stacking sequence that changes in going from one structure to the next. Thus, for the *dhcp* Cm I structure, the sequence is (A-B-A-C-A), which changes to (A-B-C-A) for the *fcc* Cm II phase by shifting planes. The *fcc* converts to an (A-B-A) in the Cm III phase by a shift and distortion of the planes. As in the orthorhombic Cm IV structure (space group *Fddd*, Cm on 8a sites), Cm III is composed of slightly distorted close-packed hexagonal planes (rectangular distortion), but in contrast to Cm IV, has a stacking that reduces the symmetry to monoclinic (the monoclinic angle is between a and c). Finally, a shift, distortion, and zigzag bending of the quasihexagonal planes yields, as in the case of Am, an (A-B-A) stacking for the Cm V phase (space group *Pnma*, Cm on 4c sites). The lattice parameters for Cm IV at 81(2) GPa are $a = 8.925(1)$, $b = 5.315(1)$, and $c = 2.737(1)$ Å (all atomic positions fixed by symmetry) and for Cm V at 100(5) GPa are $a = 4.634(1)$, $b = 4.394(1)$, and $c = 2.682(1)$ Å with atomic positions $x = 0.397$, $y = 1/4$, and $z = 0.134$.

fully delocalized. Theoretical calculations have reproduced the experimentally observed sequence of phase transitions, but only when an AFM state is assumed for Cm with 5*f* electrons throughout the first few phases. The magnetic correlations in Cm are very strong in contrast to Am in which the spin (S) and orbital (L) angular momenta of the six 5*f* electrons cancel (S = –L = 3, thus J = L + S = 0 in Russell-Saunders coupling) and there is no magnetic moment. We thus find that the magnetic correlation energy in AFM Cm plays a crucial role in establishing its structural characteristics and, in particular, in leading to the stabilization of the Cm III structure.

In only two known cases, iron and cobalt, does the energy associated with magnetic interactions influence the crystal structure of an element as a function of volume (*3–5*). The reason is that magnetic interactions are on the scale of meV, whereas structural stabilities are on the scale of eV, although at phase transitions these differences are in the meV range. Now we have found a third element, curium, which has a half-filled shell of 5*f* electrons, where this magnetic phenomenon is again observed and where the magnetic correlations of *f* electrons determine the crystal structure. The nature of the interactions and the associated crystal structure of Cm III suggest that such effects may become prevalent in other *f* elements at small atomic volumes.

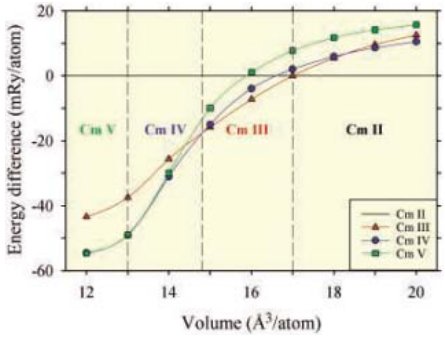

Fig. 4. Calculated ab initio total energy difference between Cm II, Cm III, Cm IV, and Cm V structures as a function of volume. The energy of the Cm II phase is taken as a reference level and is shown as a horizontal line at zero. The vertical dashed lines indicate the crossover points for each phase.

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On-Wire Lithography

Lidong Qin, Sungho Park, Ling Huang, Chad A. Mirkin*

We report a high-throughput procedure for lithographically processing one-dimensional nanowires. This procedure, termed on-wire lithography, combines advances in template-directed synthesis of nanowires with electrochemical deposition and wet-chemical etching and allows routine fabrication of face-to-face disk arrays and gap structures in the range of five to several hundred nanometers. We studied the transport properties of 13-nanometer gaps with and without nanoscopic amounts of conducting polymers deposited within by dip-pen nanolithography.

Despite their many attributes and capabilities, nanolithographic techniques such as electron-beam lithography, dip-pen nanolithography (DPN), focused ion-beam lithography, and nanoimprint lithography are limited with respect to throughput, materials compatibility, resolution, and cost (*1*). For example, the field of nanoelectronics depends on the ability to fabricate and functionalize electrode gaps less than 20 nm wide for precise electrical measurements on nanomaterials. Fabricating such structures is far from routine and often involves low-yielding, imprecise, and difficult-to-control procedures such as break-junction techniques and gap narrowing by electroplating (*2–6*). Here, we present a relatively high-throughput procedure for lithographically processing nanowires that allows us to control gap size down to the 5-nm length scale. This procedure, termed on-wire lithography (OWL), combines advances in template-directed synthesis of nanowires with electrochemical deposition and wet-chemical etching and allows the routine fabrication of architectures that would be difficult, if not impossible, to make with any known lithographic methodology (Scheme 1).

OWL is based on the idea that one can make segmented nanowires consisting of at least two types of materials, one that is susceptible and one that is resistant to wet-chemical etching. For proof of concept, we used Au-Ag and Au-Ni. We first describe the process involving Au-Ni nanowires. These materials can be electrochemically deposited in porous alumina templates (pore diameter = 360 nm) in a controlled fashion from suitable plating solutions through well-established methods (Scheme 1) (*7–11*). The length of each segment can be tailored by controlling the charge passed during the electrodeposition process (fig. S1). They are released from the template by dissolution of the template through literature procedures (*12*).

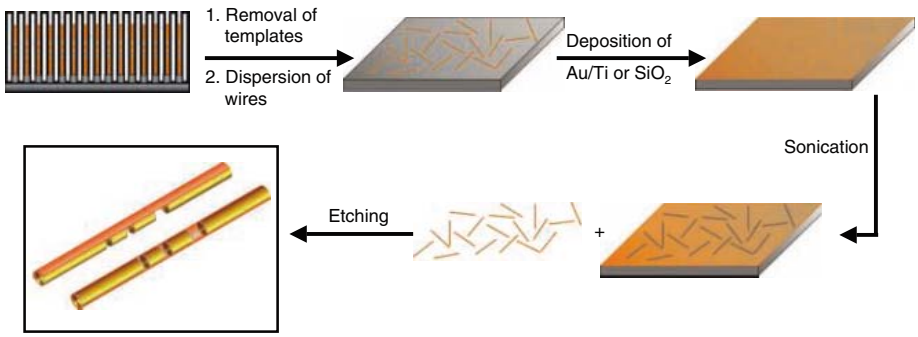
The nanowire aqueous suspension was cast on a glass microscope slide, pretreated with piranha solution to make it hydrophilic, and allowed to dry in a dessicator. A layer of silica (50 nm) was deposited on the nanowire-coated substrate by plasma-enhanced chemical vapor deposition (*13*). The substrate was immersed in ethanol and sonicated (VWR Ultrasonic Cleaner, MODEL 50T) for 1 min, which resulted in the release of the wires (fig. S2).

The final step of the OWL process involves the selective wet-chemical etching of the Ni segments. The Ni can be removed from the wires by treating them with concentrated HNO_3 for 1 hour to generate nanowire structures with gaps precisely controlled by the length of the original Ni segments (Scheme 1).

We also prepared similar structures using Ag as the sacrificial segment material and an Au/Ti bilayer (40 nm/10 nm) deposited by thermal evaporation as the segment-bridging layer instead of silica. In this case, the Ag was removed by treating the wires with an etching solution consisting of methanol, 30% ammonium hydroxide, and 30% hydrogen peroxide (4:1:1 v/v/v) for 1 hour.

Using the OWL procedure, we prepared nanowires with designed gaps of 5, 25, 40, 50, 70, 100, 140, and 210 nm (Fig. 1). The physical dimensions and block compositions of the nanowires, before and after etching, were determined by field-emission scanning electron microscopy (FESEM) and energy-dispersive x-ray spectroscopy (fig. S3), respectively. Structures made of Au and Ag before coating with Au/Ti and wet-chemical etching exhibit a bright contrast for the Au regions and a dark contrast for the Ag regions (Fig. 1A). Etching then creates the notched structures (Fig. 1B). The average length of the wires (±SD) is 4.5 ± 0.25 μm, and each wire exhibits two notches measuring 210 ± 10 nm, two measuring 140 ± 8 nm, and two measuring 70 ± 5 nm (Fig. 1B). The diameter of each wire is 360 ± 20 nm. Certain views show the Au/Ti backing, which bridges the notched regions on these structures (Fig. 1B, inset). Structures with gap sizes of less than 100 nm can be routinely generated with OWL. To demonstrate this capability, we used OWL to prepare wires with 25-, 50-, and 100-nm gaps (Fig. 1C).

We observed similar results with the use of Ni as the sacrificial segment and silica as the bridging material. To demonstrate that OWL can make repeating structures consisting of regular 40-nm gaps, we made nanowire structures with 22 40-nm Ni segments and 23 40-nm Au segments (Fig. 1D). After coating with silica and subsequent removal of the Ni blocks, face-to-face disk arrays with 40-nm gaps were generated (Fig. 1E). The statistical



Scheme 1. OWL methodology.

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variation of gap size generally increases with decreasing gap size but is typically less than 10%. The greater variation in some images is caused by mechanical stress on the wire structures, which results in a "fanning" effect with respect to the gaps. The smallest gap structures generated to date with the OWL approach are 5 nm (Fig. 1F), but with the appropriate electrochemical control, there is no reason this number cannot be reduced.

An important issue involving the characterization and utility of gaps fabricated by OWL pertains to their transport properties. In principle, for the structures prepared with the silica bridging material, one can use the micrometer-scale gold ends as electrode leads that can be interfaced with larger microelectrode electronic circuitry. Indeed, all of the structures with gaps have been characterized by current versus voltage (I-V) measurements and exhibit insulating behavior (green line in Fig. 2A). To test the properties of these structures and their suitability for making transport measurements on small amounts of materials contained within the gaps, we evaporated a droplet of a suspension that contained wires with 13-nm gaps on a microelectrode array fabricated by conventional photolithography (Fig. 2, B and C). The electrodes were 3 μm wide and separated by 2 μm. Some of the wires ended up bridging the microelectrodes, allowing us to easily make electrical measurements on such structures.

Measurements of I-V curves show that the gaps within the nanowires are insulating (green line in Fig. 2A). The gaps within the nanowires can be functionalized with many materials in a site-specific manner using DPN (fig. S4) (*14*, *15*). As proof of concept, we deposited a mixture of polyethylene oxide and self-doped polypyrrole (PEO:PPy = 1:1 w/w) into the gap by DPN. This approach allows us to monitor the device architecture in the active region, measure the topography of the nanowires, and simultaneously functionalize the nanowire gaps with molecule-based materials. An SEM image after modification of the gap with polymer shows the clear contrast between the clean gold surface and the polymer-covered area including the gap (Fig. 2C, inset). After deposition of the polymer, the I-V curves show a linear response from –1.0 to 1.0 V, characteristic of the conducting polymer (red line in Fig. 2A). The measured conductance of 1.1 nS is similar to the value of 9.6 nS determined by functionalizing 60-nm conventional nanoelectrode gaps fabricated by electron-beam lithography in our lab. There is no noticeable I-V hysterisis between the forward (from –1.0 to +1.0 V) and backward (from +1.0 to –1.0 V) scans, and they are highly linear at room temperature, as expected for a structure with an ohmic-like contact in a symmetric device configuration.

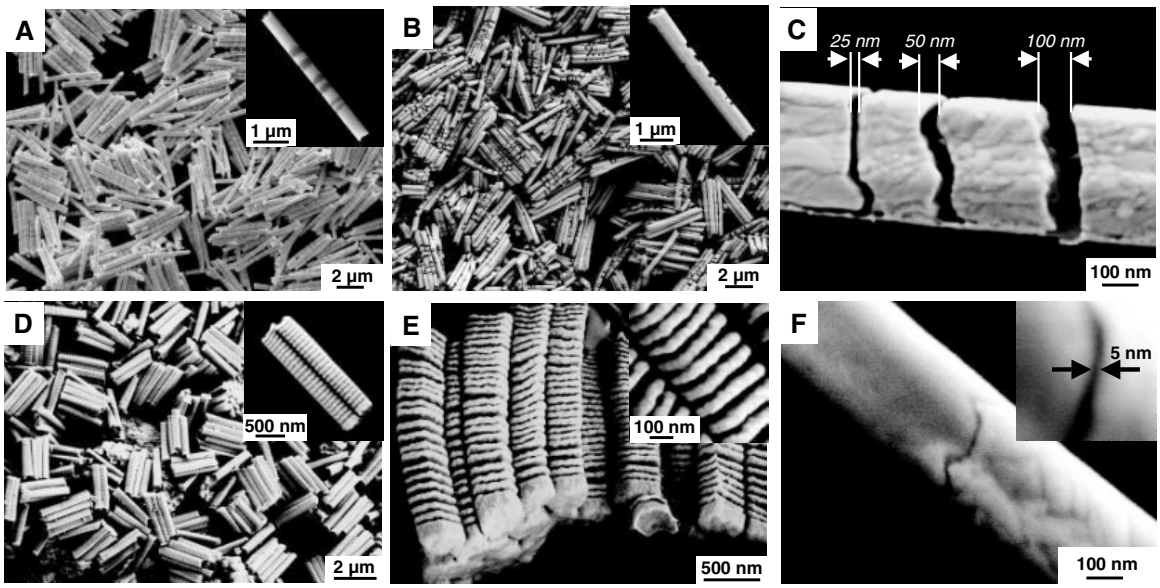

Fig. 1. FESEM images of multisegment metallic Au-Ag nanowires (**A**) before and (**B**) after coating with a bilayer consisting of 10 nm of Ti and 40 nm of Au and subsequent wet-chemical etching of the Ag segments. Insets show magnified images. (**C**) Side view of a nanogap wire with gap sizes of 25, 50, and 100 nm. Multisegment metallic Au-Ni nanowires (**D**) before and (**E**) after coating one side with 50 nm of silica and subsequent wet-chemical etching of the Ni segments. Each Au and Ni segment length is 40 nm. Insets are magnified images. (**F**) An Au nanowire with a 5-nm gap. Every nanowire in the batch has a well-defined gap.

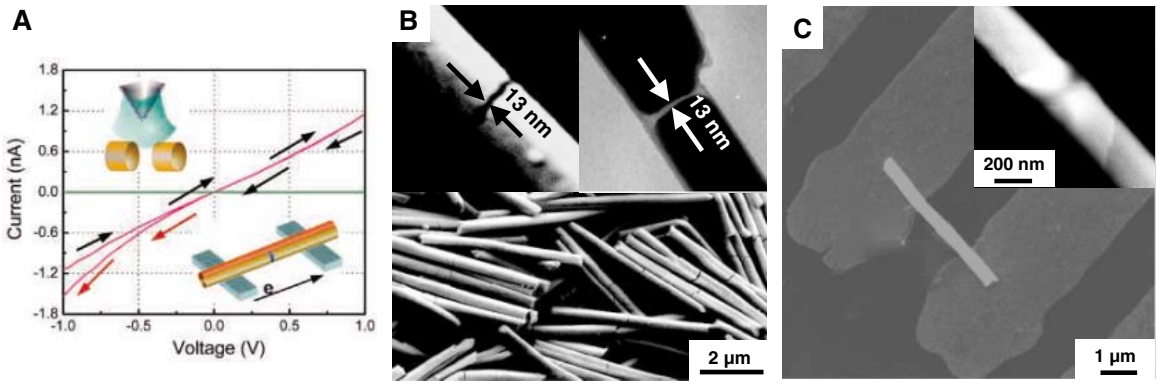

Fig. 2. (**A**) Current-voltage measurements on the wires with nanogaps. The green line is before functionalization of the gap with PPy/PEO by DPN, and the red line is after it has been filled with the polymer mixture. The inset describes the DPN process. The red arrows indicate the observed conductivity change under Xe light irradiation. e, electrons. (**B**) Images of Au wires with 13-nm gaps. In the center of each wire, the dark lines are the nanogaps. The contrast depends on which side is facing out because of the silica coating. The left inset is a magnified FESEM image of a 13-nm gap, and the right inset is a magnified transmission electron microscopy image, which shows the transparent gap. (**C**) An FESEM image of a wire with a 13-nm gap, which is immobilized on microelectrodes and filled with PPy/PEO. (Inset) An image that shows clear contrast between the clean gold surface and the polymer-covered area, including the gap. The contrast is a result of differences in substrate charging due to the presence of organic polymer.

To show that the response is indeed from the polymer within the gap, we studied the I-V response as a function of photoexcitation with a Xe lamp (150 W). The I-V response for the polymer-filled nanowire becomes slightly more conductive upon Xe light exposure. During the backward scan, the device was irradiated with the Xe lamp starting at –0.1 V (red arrows in Fig. 2A), and a change in slope in the I-V response was observed. The transient conductance change between 1.1 nS in the dark to 1.6 nS when irradiated is consistent with an increase in charge-carrier density, which would be expected if the gap were filled with the p-type polypyrrole (*16*).

We report a novel lithographic process that allows one to generate designed gap structures on nanowire templates. The process is remarkably controllable, high-yielding, and easy to implement. It does not require sophisticated and expensive instrumentation and facilities, and it allows manipulation of an important class of structures that cannot be easily manipulated with conventional lithographic tools. Being able to make gap or notched structures with nanowires with OWL and relatively inexpensive instrumentation will facilitate the study of the electronic properties of nanomaterials and open avenues to the preparation of novel disk structures, which could be designed to have unusual optical properties as a function of gap and metal segment size [e.g., plasmon waveguides (*17*)].

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Atlantic Ocean Forcing of North American and European Summer Climate

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Recent extreme events such as the devastating 2003 European summer heat wave raise important questions about the possible causes of any underlying trends, or low-frequency variations, in regional climates. Here, we present new evidence that basin-scale changes in the Atlantic Ocean, probably related to the thermohaline circulation, have been an important driver of multidecadal variations in the summertime climate of both North America and western Europe. Our findings advance understanding of past climate changes and also have implications for decadal climate predictions.

Instrumental records show that during the 19th and 20th centuries, there were marked variations on multidecadal time scales in the summertime climate of both North America (*1–4*) and western Europe (*5*). In the continental United States, there were significant variations in rainfall and drought frequency (*1–4*), and it has been suggested (*1*, *4*) that changes in the Atlantic Ocean, associated with a pattern of variation known as the Atlantic Multidecadal Oscillation (AMO) (*6*, *7*), were responsible. If confirmed, such a link would be important for climate predictions because the AMO is thought to be driven by the ocean's thermohaline circulation (*6*) and may be predictable (*8*, *9*). However, thus far the evidence for an Atlantic link is mainly circumstantial, being derived from observations and showing correlation rather than causality. Clarifying whether AMO-related changes in the Atlantic Ocean were indeed responsible for the observed variations in North American summer climate and whether, in addition, there were impacts on other regions is therefore an important challenge.

Figure 1 shows the time series and pattern of North Atlantic sea surface temperatures (SSTs) that characterize the AMO during the period 1871 to 2003 (*10*). There are AMO warm phases in the late 19th century and from 1931 to 1960; cool phases occur from 1905 to 1925 and from 1965 to 1990. The spatial pattern shows anomalies of the same sign over the whole North Atlantic, with the largest anomalies ($\sigma \sim 0.3$°C) found just east of Newfoundland.

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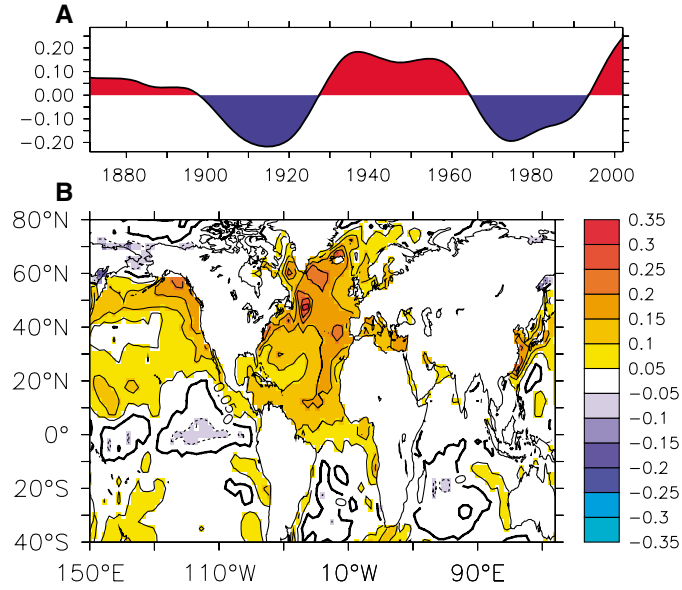

Fig. 1. (**A**) Index of the AMO, 1871 to 2003. The index was calculated by averaging annual mean SST observations (*29*) over the region 0°N to 60°N, 75°W to 7.5°W. The resulting time series was low-pass filtered with a 37-point Henderson filter and then detrended, also removing the long-term mean. The units on the vertical axis are °C. This index explains 53% of the variance in the detrended unfiltered index and is very similar to that shown in (*1*). (**B**) The spatial pattern of SST variations associated with the AMO index shown in (A). Shown are the regression coefficients (°C per SD) obtained by regressing the detrended SST data on a normalized (unit variance) version of the index.

To identify the climate variations associated with the AMO, we considered a simple composite difference of observational sea-level pressure (SLP), precipitation, and surface air temperature (SAT) data between the warm phase from 1931 to 1960 and the subsequent 30 years, 1961 to 1990, which were dominated by a cool phase of the AMO (Fig. 2, A to C). In the North Atlantic region, there are two prominent low-pressure anomalies, one centered over the southern United States (~60 Pa) and the other centered just west of the United Kingdom (peaking at ~150 Pa). The low-pressure anomaly over the southern United States is associated with precipitation reductions of up to 20% (0.1 to 0.3 mm/day), consistent with (*1*) and (*4*). Over western Europe, there is enhanced precipitation (0.1 to 0.3 mm/day, or 5% to 15% of the mean summer value). This multidecadal change in European precipitation has been previously documented (*5*) but has not been linked with the AMO. The SAT fields (Fig. 2C) show warm anomalies (0.25°C to 0.75°C) over the United States and also over central Europe. The precipitation fields (Fig. 2B) also show large positive anomalies in the Sahel region of North Africa, consistent with earlier work (*11*), and in the Caribbean.

A simple significance test (*12*) suggests that the major observed anomalies shown in Fig. 2 are unlikely to have arisen from internal fluctuations of the atmosphere. To investigate whether they arose in response to changes in the ocean, we first examined results from an ensemble of six simulations with an atmospheric general circulation model. These "C20" simulations were forced with historical global SST data for the period 1871 to 1999, and variations in the ensemble mean provide information about the ocean forcing of climate (*13*). Figure 2, D and E, shows the ensemble mean SLP and precipitation anomalies corresponding to the composite differences computed from observations. The SLP shows low-pressure anomalies centered over the southern United States and in the region of the United Kingdom that are in good agreement with the observations. The anomalies over the United States are very similar in magnitude to the observations (60 to 75 Pa), whereas the anomalies west of the United Kingdom are weaker in the model ensemble mean (~50 Pa) than in the observations (~150 Pa). The most likely reason for this discrepancy is a larger component of internal variability in the observations, a hypothesis supported by analysis of the ensemble spread (fig. S3).

The precipitation field (Fig. 2E) shows reduced precipitation over the United States and northern Mexico, and the magnitude of the anomalies (0.1 to 0.3 mm/day) is in agreement with the observations. Over western Europe, the model indicates enhanced precipitation consistent with the observations, but on the basis of our sample of six ensemble members, the anomalies are not statistically significant and are therefore not seen in the figure. The model shows large increases in precipitation in a tropical band stretching from the eastern Pacific, through the Caribbean and tropical Atlantic, to North Africa. The anomalies in the Caribbean and northern South America agree with the land observations in these regions, but the anomalies in the Sahel region are markedly weaker than is observed. This discrepancy might be a consequence of errors in the representation of land-surface feedbacks (*14*, *15*).

The results from the C20 simulations provide strong evidence that the major North Atlantic features identified in the observations arise in response to changes in the oceans. Results from similar experiments with other atmospheric models are consistent with this conclusion (*16*). However, because in each case the model was forced with global SST fields, these experiments do not clearly demonstrate the role of the Atlantic Ocean. To clarify the role of the Atlantic, we forced the model with an idealized AMO SST anomaly pattern, based on the North Atlantic part of Fig. 1B (see fig. S1). For these experiments, SST anomalies did not vary in time, but integrations of 10 or 20 years' duration were carried out to separate the ocean's influence from atmospheric internal variability (*12*).

Figure 2, F to H, shows the response to the AMO SST pattern. Over the Caribbean, central America, and the United States, the SLP and precipitation fields show excellent agreement with both the C20 simulations and the observations. The low-pressure anomalies in western Europe are also reproduced. Over the United Kingdom, there are positive precipitation anomalies, which were not seen in the C20 simulations but are in the observations, whereas precipitation anomalies over the Sahel are again weaker than observed (*17*). The SAT fields (Fig. 2H) show a prominent warm anomaly

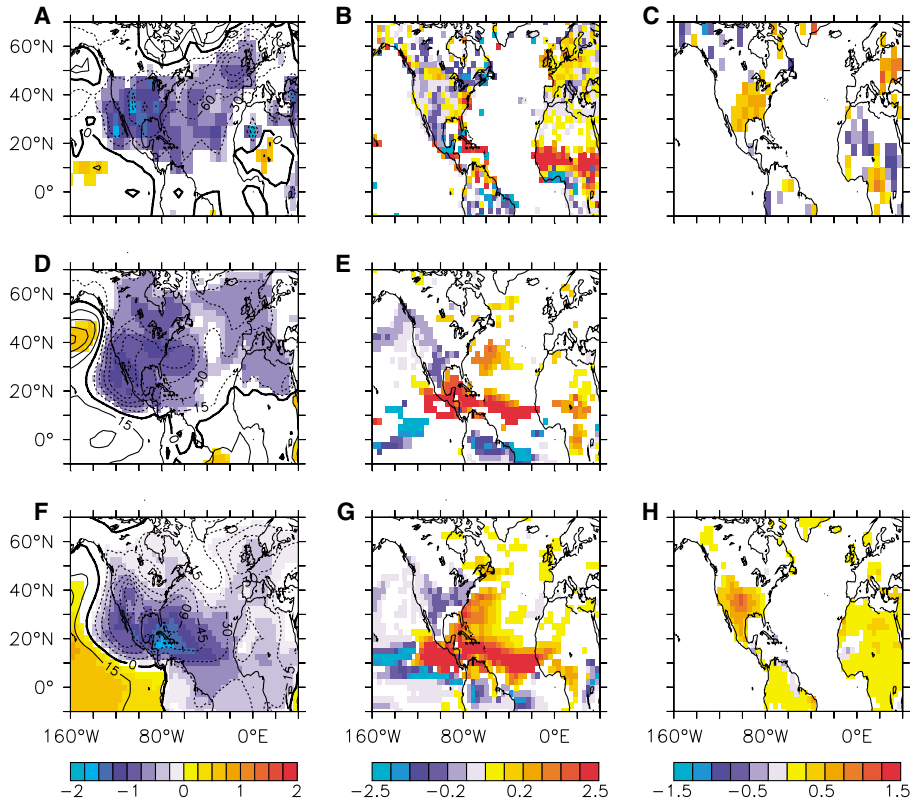

Fig. 2. Evidence of AMO impacts on boreal summer [June, July, and August (JJA)] climate. (**A** to **C**) Observed differences between the mean JJA conditions from 1931 to 1960 (a warm phase of the AMO) and the mean JJA conditions from 1961 to 1990 (a cold phase of the AMO). (A) Sea-level pressure. Contours are in Pa with an interval of 30 Pa; shading indicates signal-to-noise ratio (*12*). (B) Land precipitation (mm/day). (C) Land surface air temperature (°C). The scale for precipitation is nonlinear; the central range is (–0.5, 0.5). Values between (0.5,2.5) and (–2.5,–0.5) are each shaded with a single color. (**D** and **E**) As in (A) and (B), but computed from the ensemble mean of six simulations with the HadAM3 atmosphere model forced with observed SST data. In (D), the contour interval is 15 Pa. (**F** to **H**) As in (A) to (C), but showing differences between time means of simulations with the HadAM3 model forced with positive and negative signs of an idealized AMO SST pattern. (The pattern is based on the North Atlantic part of Fig. 1B and is shown exactly in fig. S1.) In (F), the contour interval is 15 Pa. All the values have been appropriately scaled to allow comparison with the other panels (*12*). In (A) and in (C) to (H), regions where anomalies are not significant at the 90% level are shaded white. In (E) and (G), precipitation values are shown over the sea as well as the land. Details of the model experiments and analyses are given in (*12*).

over the southern United States and Mexico, in agreement with the observations, and also show positive anomalies in western Europe. Overall, Fig. 2 provides compelling evidence that the AMO has indeed been responsible for marked changes in the regional atmospheric circulation and for associated anomalies in precipitation and surface temperature over the United States, southern Mexico, and, probably, western Europe.

The fact that the AMO SST experiments and the C20 simulations give such similar results suggests that, for the particular decadal change considered (1931 to 1960 compared with 1961 to 1990), the Atlantic Ocean was the dominant oceanic influence on summertime climate in the regions considered. To investigate the importance of the AMO over a longer period of time, we examined the correlation between the AMO SST index (Fig. 1A) and indices of SLP for the U.S. and U.K. regions (*12*). The SLP indices were computed both from the observations and from the C20 simulations. All the SLP indices are significantly anticorrelated with the AMO index (fig. S2). These results confirm that the AMO is an important influence on both regions, but they also suggest that other influences are important, especially for the U.S. region (for which the correlation with observed SLP is lowest). The much higher correlation between the AMO index and simulated U.S. SLP could suggest that these other influences [e.g., associated with changes in the Pacific Ocean (*3*)] are underestimated in the HadAM3 simulations.

To understand better the AMO influence, we performed two additional model experiments, using as forcing patterns the tropical part (0°N to 30°N) and the extratropical part (30°N to 70°N) of the AMO SST pattern (see fig. S1). The results (Fig. 3) indicate that AMO-related climate anomalies over the United States and Mexico were forced by tropical Atlantic SST anomalies, whereas those in the region of western Europe were primarily (but not exclusively) forced by SST anomalies in the extratropics. The low-pressure center over the southern United States may be a response to the anomalous latent heating of the atmosphere implied by the enhanced precipitation in the tropical Atlantic (*18*). The low-pressure center near the United Kingdom may be a downstream response to the largest positive SST anomalies east of Newfoundland (*19*, *20*). In Fig. 3, A and B, the impacts of the AMO are not restricted to the Atlantic basin but extend throughout the tropics. This finding supports suggestions (*21*, *22*) that the Atlantic Ocean may be an important driver of multidecadal climate variability on a global, as well as regional, scale.

Overall, our results provide strong evidence that during the 20th century the AMO had an important role in modulating boreal summer climate on multidecadal time scales. We have focused here on time mean anomalies, but some of the most important impacts are likely to be associated with changes in the frequency of extreme events. There is evidence that the frequency of U.S. droughts (*4*) and the frequency of European heat waves (*23*) are both sensitive to Atlantic SSTs.

The results also have implications for the interpretation of instrumental and proxy (*24*) climate records, which relies on understanding and quantifying how regional climate is related to large-scale drivers such as the AMO. Progress in understanding these drivers is especially important as studies that address the attribution of observed climate changes to anthropogenic causes give increasing attention to regional scales (*25*). Our results suggest, for example, that the change in phase of the AMO in the 1960s may have caused a cooling of U.S. and European summer climate; a further change in the AMO may have contributed to recent warming in these regions.

In addition, our results have implications for predicting the climate of the next few decades. In the absence of anthropogenic effects and assuming a period of 65 to 80 years (*1*, *26*), we should now be entering a warm phase of the AMO, as suggested by Fig. 1A. Our results would then suggest a forecast of decreased (relative to 1961 to 1990) summer precipitation (increasing drought frequency) and warmer temperatures in the United States together, possibly, with increased summer precipitation and temperatures in western Europe. In reality, anthropogenic effects on climate now appear to be important (*27*), so current and near future trends in North Atlantic regional climate may be shaped by a competition between the AMO and these anthropogenic effects. Moreover, these two influences may not add linearly. Models suggest that anthropogenic warming will lead to a slowdown of the Atlantic thermohaline circulation (*27*). If true of the real world, this could favor an earlier than expected shift toward the negative phase of the AMO, with a corresponding shift in the AMO influences on U.S. and European summer climate.

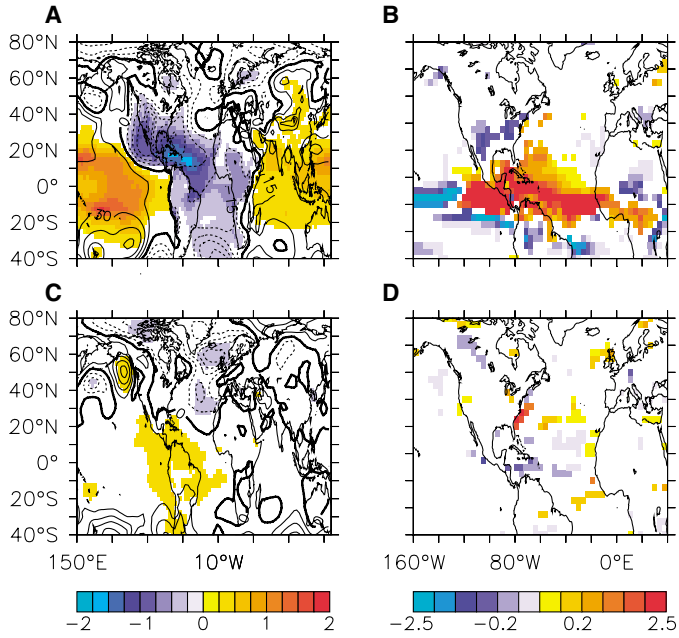

Fig. 3. Response of the HadAM3 model to tropical North Atlantic (TNA) and extratropical North Atlantic (XNA) parts of the AMO SST pattern. (**A** and **B**) Differences between time means of simulations with the HadAM3 model forced with positive and negative signs of the TNA SST pattern. (A) Sea-level pressure. Contours in Pa with an interval of 15 Pa; shading indicates signal-to-noise ratio (*12*). (B) Precipitation (mm/day); scale is nonlinear as described for Fig. 2. (**C** and **D**) As in (A) and (B), but for model simulations forced with the XNA SST pattern. The TNA and XNA SST patterns are shown in fig. S1. All values have been scaled to allow comparison with the Fig. 2 results. Regions where anomalies are not significant at the 90% level are shaded white.

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GRIP Deuterium Excess Reveals Rapid and Orbital-Scale Changes in Greenland Moisture Origin

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The Northern Hemisphere hydrological cycle is a key factor coupling ice sheets, ocean circulation, and polar amplification of climate change. Here we present a Northern Hemisphere deuterium excess profile covering one climatic cycle, constructed with the use of $\delta^{18}O$ and δD Greenland Ice Core Project (GRIP) records. Past changes in Greenland source and site temperatures are quantified with precipitation seasonality taken into account. The imprint of obliquity is evidenced in the site-to-source temperature gradient at orbital scale. At the millennial time scale, GRIP source temperature changes reflect southward shifts of the geographical locations of moisture sources during cold events, and these rapid shifts are associated with large-scale changes in atmospheric circulation.

The atmospheric water cycle plays a key role in climate change. At various time scales, changes in atmospheric moisture transport are intimately involved in key processes within the climate system, such as the growth of ice sheets or the freshwater budget of the ocean. Here we use an integrated tracer of the water cycle, the isotopic composition of the ice preserved in Greenland, to decipher changes in Greenland moisture origin over the last glacial cycle.

Water stable isotopes ratios ($\delta^{18}O$ or δD) from polar ice cores are commonly used as past temperature proxies (*1*, *2*), a function made possible by the progressive distillation of heavy water isotopes when air masses cool toward polar regions (*3*–*5*). Comparison of Greenland temperature (T_{site}) values derived from ice $\delta^{18}O$ using the observed modern spatial gradient with alternative paleothermometry methods yields a systematic underestimation of past surface annual mean temperature changes, both at glacial-interglacial (*6*–*8*) and rapid-events time scales (*9*–*13*). Such discrepancies are thought to arise from variability in the Northern Hemisphere hydrological cycle, either from changes in moisture source areas (*14*) or from changes in the seasonality of precipitation (*15*, *16*).

We used high-precision continuous water stable isotope measurements made on the GRIP ice core (table S1) to calculate a Northern Hemisphere deuterium excess $d = [\delta D - (8 \times \delta^{18}O)]$ profile covering one climatic cycle (*17*). This deuterium excess record, together with a method to account for changes in precipitation seasonality, was then used to quantify past changes in Greenland moisture source temperature.

Figure 1 shows both $\delta^{18}O$ and deuterium excess profiles for the last ~100,000 years (i.e., excluding the lowest part of the profiles characterized by ice flow disturbances) (*18*). The excess profile reveals well-defined features at glacial-interglacial time scales, with a 5 per mil (‰) increase during the last climatic transition (*5*), as well as for Dansgaard-Oeschger (D/O) events characterized by large (up to 5‰) excess changes, in antiphase with $\delta^{18}O$ changes. Similar rapid excess changes are also recorded in the North Greenland Ice Core Project (NorthGRIP) ice core for D/O events 18 to 20 (*13*). Such deuterium-excess variations essentially result from the fact that, with respect to equilibrium processes, kinetic isotopic effects play a much larger relative role for $\delta^{18}O$ than for δD. In turn, deuterium excess in polar snow, d_{snow}, is largely driven by nonequilibrium processes (i.e., evaporation at the ocean surface and condensation of water vapor when snow forms). Deuterium excess in water vapor over the ocean is mainly influenced by sea surface isotopic composition, sea surface temperature (SST), source temperature (T_{source}), and relative humidity. This imprint of oceanic conditions (here considered only in terms of T_{source}) in the moisture source areas is largely preserved in the deuterium excess signal recorded in polar snow (*19*). In turn, whereas δ_{snow} ($\delta^{18}O$ or δD) depends primarily on local temperature T_{site} and to a lesser degree on T_{source}, one can show that the opposite is true for d_{snow} (*20*, *21*).

Combining $\delta^{18}O$ and deuterium excess profiles allows us to estimate both T_{site} and T_{source}. This dual approach, based on the inversion (*22*) of a dynamically simple isotopic model (*23*), is now used for interpreting isotopic measurements performed on Antarctic cores (*24*–*26*). Although useful for the last millennium and for the Holocene in Greenland (*20*, *27*), this methodology leads to unrealistic results when applied directly to the long-term GRIP data (*21*). We show here that this difficulty can be overcome if this inversion accounts for the seasonality of snow precipitation, as suggested by general circulation model simulations. This allows us to interpret the $\delta^{18}O$ or δD GRIP data in terms of T_{site} and T_{source} changes in a consistent way, both for glacial-interglacial changes and for D/O events, thus providing high-resolution information on the source conditions and the reorganizations of the water cycle during slow and rapid climatic changes.

Seasonal characteristics are relatively well known for central Greenland's present-day climate. Field observations suggest a year-

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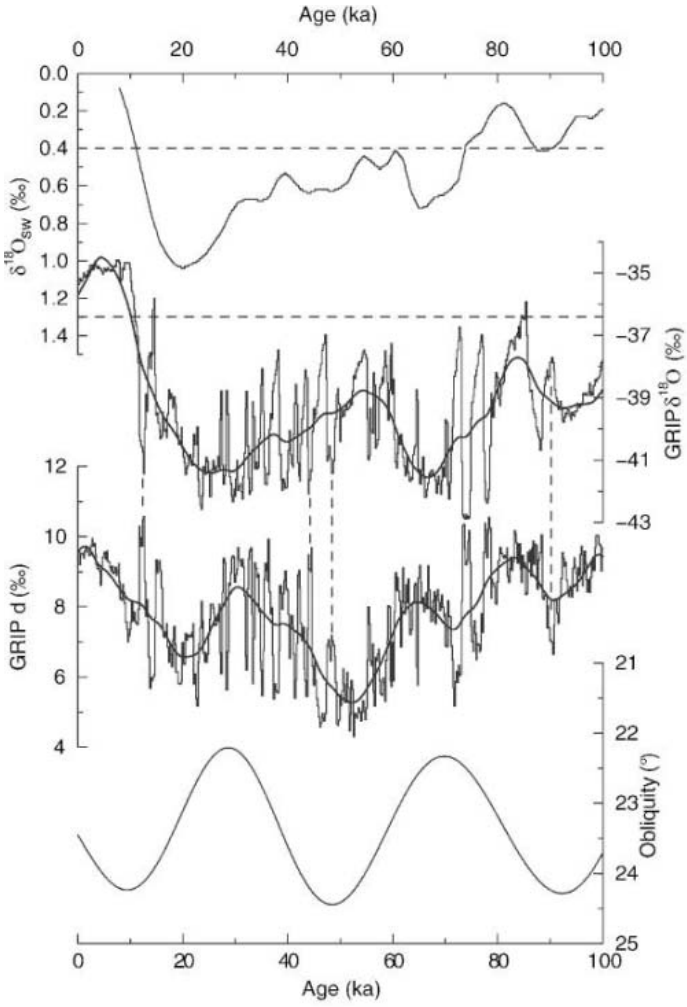

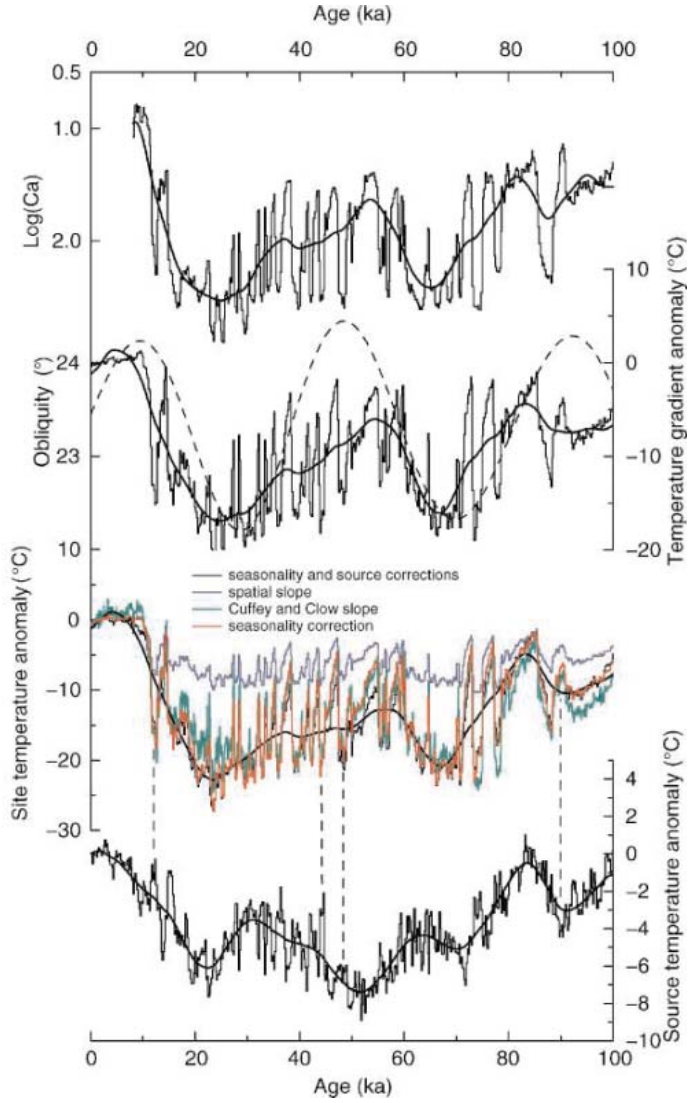

Fig. 1 (**left**). From top to bottom: ice volume indicated by seawater $\delta^{18}O_{sw}$ (*31*); 200-year resampled GRIP $\delta^{18}O$ and deuterium excess *d* (bold lines represent isotopic profiles smoothed using the first two components of a singular spectral analysis, which represents a low-pass filter below 10,000 years); and obliquity (*43*). Analytical precisions are ±0.05‰ for $\delta^{18}O$ and ±0.50‰ for δD, resulting in a precision of ±0.64‰ for deuterium excess. Horizontal dashed lines identify thresholds T_1 and T_2 used for the seasonality correction. Vertical lines point to selected rapid events (from left to right: Younger Dryas, cold phases of D/O events 11, 12, and 22). **Fig. 2** (**right**). From top to bottom: log(Ca) (*39*); 200-year reconstructed site-to-source temperature gradient (black solid line) and obliquity fluctuations (black dashed line); site temperature (ΔT_{site}) reconstructed using various methods [spatial slope without seasonality correction, blue; our inversion with seasonality correction but without source correction, red; our full isotopic inversion with seasonality correction, black; the use of a constant slope fitting with the LGM borehole estimate (*32*), green]; source temperature (ΔT_{source}) reconstruction from full inversion. Results are displayed as anomalies from modern conditions (present-day GRIP site temperature is ~ −32°C and GRIP source temperature is ~20°C). Bold lines represent smoothed reconstructions as for Fig. 1.

round snowfall at Summit with a slightly larger winter half-year accumulation due to intense episodic storms (*28*). The seasonal variations are strongly imprinted in $\delta^{18}O_{snow}$, which closely follows the temperature changes. As shown by detailed shallow ice core and pit studies, there is also a well-documented d_{snow} seasonal cycle that lags variations in temperature, δD, and $\delta^{18}O$ by ~2 to 3 months (*5*, *29*). This delay is probably due to the thermal inertia of the ocean, which results in a 2- to 3-month lag between mid-latitude ocean surface temperature and continental surface air temperature.

In contrast, glacial conditions inhibit winter snowfall because of the modification of the stationary waves and the southward deviation of the winter storm tracks by (i) the Laurentide ice sheet, and (ii) the modified latitudinal ocean surface temperature gradients and increased extent of sea ice (*30*). Modeling results suggest a large change in precipitation seasonality toward a dominant summer contribution (*15*, *16*). We also expect an enhanced δ_{snow} seasonal amplitude due to increased glacial Greenland continentality. The ~2- to 3-month lag of the deuterium excess cycle, thermally driven, should be essentially unchanged.

A detailed sensitivity analysis shows that a simple half-year (summer-winter) approach is well adapted to account for these seasonality changes for reconstructing site and source temperature records (*21*). Whereas the winter-summer difference is large for δ_{snow}, it is small (less than 1‰) for the deuterium excess because of its seasonal lag. Hence, seasonality has a strong impact on estimates of T_{site} but a much weaker influence on T_{source}, as these two variables are driven by δ_{snow} and d_{snow}, respectively. The glacial decrease in winter precipitation results in amplified seasonally corrected isotopic signals by a factor that is also influenced by the changes in the summer-winter isotopic amplitude [(*21*), equation 5]. We account for seasonality when two conditions linked with sea-ice extent and Laurentide ice sheet size are fulfilled: (i) Sea-ice extent is assumed to inhibit local moisture supply when the site temperature is below a certain threshold T_1 directly derived from the ice $\delta^{18}O$ record, and (ii) to reflect the fact that the winter moisture advection is related to the size

of the Laurentide ice sheet, we account for seasonality changes only when the Laurentide is large enough. We use here a second threshold, T_2, derived from the marine benthic $\delta^{18}O$ sea level record, itself closely related to the size of the Laurentide (*31*). Ensemble simulations of seasonality correction and full isotopic inversion are then performed within a wide range of correction coefficients. Obtaining a consistency with robust estimates of site temperature amplitudes obtained with independent methods (Table 1) for the Last Glacial Maximum (LGM), Younger Dryas, Bølling-Allerød, and D/O events 12 and 19 constrains the thresholds T_1 ($\delta^{18}O$ of ice < –36.4‰) and T_2 (ocean $\delta^{18}O$ < 0.4‰), and also the amplification coefficient, to a value of 2.0.

The resulting site and source fluctuations are shown in Fig. 2, showing a warming of ~21.5°C from the LGM to the Holocene [estimate based on the 2000-year period centered at 21,000 years ago (21 ka)]. Our reconstruction for D/O events 18 and 20 is also consistent with independent estimates of site temperature changes obtained using gas fractionation (performed in this latter case on the NorthGRIP core located 300 km north-northwest of the GRIP site) (Table 1). Figure 2, which compares various approaches to reconstructing T_{site} changes from water isotopes, clearly shows the importance of accounting for seasonality. However, there is also a significant influence of the source temperature (full inversion, black curve), which results in a systematic shift of the warm part of each D/O event toward colder temperatures. This is due to the antiphase between the $\delta^{18}O$ (or δD) and excess rapid variations: When Greenland is warm, the moisture source is colder, and if this is not corrected for, temperature estimates will be too cold (and vice versa). Neglecting changes in moisture source and using a constant temporal isotope-temperature slope of half the spatial slope (*32*) is therefore inadequate to reconstruct past Greenland temperatures from $\delta^{18}O$ and could induce an overestimation of some rapid-events temperature change by up to 40% (Fig. 2 and Table 1). In other words, the apparent isotope-temperature slope varies at all time scales (*13*).

Source temperature changes, ΔT_{source} (Fig. 2), mimic the initial excess record (Fig. 1) with a glacial-interglacial amplitude of ~6°C. Rapid T_{source} changes of 2° to 4°C occur simultaneously but in antiphase with rapid T_{site} events, which is remarkable given that these two climate variables appear in phase at longer orbital scales; the large amplitude of rapid events in deuterium excess is partly due to large changes in Greenland temperature itself (*21*) and is less marked after isotopic inversion in T_{source}. As is the case for Antarctica, an imprint of obliquity fluctuations in deuterium excess and source temperature can be seen, although this is limited to the period from 20 to 80 ka. The mechanisms at work are probably the same as in Antarctica. In low latitudes, a low obliquity is associated with a high local mean annual insolation, which should result in higher SST [as recently suggested by a modeling experiment (*33*)] and thus in a more intense evaporation. A low obliquity also implies a decrease in high-latitude insolation and temperature (*34*). The resulting increased insolation gradient is associated with a more intense meridional temperature gradient (Fig. 2) and a more intense atmospheric transport (*35*). These two effects together yield a dominant role for warm low-latitude sources when obliquity is low, thus explaining the observed link between deuterium excess and obliquity. However, such a link is not observed between 20 and 10 ka and before 80 ka, possibly because these two periods—which correspond to large ice sheet changes (deglaciation and glacial inception, respectively)—are characterized by changes in Northern Hemisphere stationary waves forced by ice sheet growth or decay. Finally, high obliquities (e.g., at 30 and 70 ka) correspond to a high polar amplification (ratio of long-term changes in T_{site} to T_{source} up to 6), whereas periods with low obliquity (e.g., at 10, 50, and 90 ka) coincide with a reduced polar amplification (ratio of change around 2).

North Atlantic SST records (*36*) show large glacial-interglacial fluctuations, with thermal amplitudes reaching typically ~10°C at 45°N and ~5°C at 35°N (*37*), significantly higher than the glacial-interglacial source temperature change reconstructed here. A detailed inspection of ΔT_{source} (Fig. 2), which resembles the initial deuterium excess record (Fig. 1), also points to important differences at millennial time scales. High-resolution North Atlantic SST records exhibit rapid changes generally in phase with Greenland D/O events recorded in T_{site}, whereas ΔT_{source} reconstruction suggests an out-of-phase relation between T_{source} and T_{site} during rapid changes.

Our proposed interpretation is that these noticeable differences (amplitude of LGM-to-modern changes and millennial-scale events) result from drastic changes in the polar front, the geographical location of Greenland's main moisture sources, and the atmospheric water cycle during glacial times (*5*). When Greenland is cold and surrounded by an extensive sea ice cover, mid-latitude oceans are much colder than today and cannot provide much moisture to Greenland. Evaporation is, however, maintained at lower subtropical and tropical locations, providing precipitation in central Greenland largely in summer. Typically, a southward moisture source shift of 5° in latitude is compatible both with the LGM ΔT_{site} values and with local summer SST reconstructions. This is also consistent with temperature and salinity latitudinal profiles estimated from marine sediments, reflecting the shift of the dominant evaporative areas during rapid events (*38*).

Such a large-scale reorganization of the hydrological cycle at both orbital and millennial time scales is indirectly confirmed by the calcium composition of GRIP ice, a parameter reflecting (i) the strength of GRIP dust sources mainly provided by Chinese loess areas, and (ii) the efficiency of dust transport to Greenland (*39*). The strong correlation between log(Ca) and $\delta^{18}O$ of ice was previously noted (*39*), but the similarity is even better with this reconstructed site-to-source temperature gradient (R^2 = 0.86 at 100-year time step and R^2 = 0.88 at 5000-year time step between 8 and 100 ka). In particular, a warmer site and a colder source at ~50 to 55 ka result in a decreased meridional temperature gradient, exactly at the time of minimum calcium concentration. The meridional temperature gradient also shows a 40,000-year modulation paralleling obliquity fluctuations (Fig. 1). We have already pointed out that mean annual insolation, and thus obliquity, strongly controls the meridional atmospheric circulation. We suggest that they also indirectly control the strength of the dust source: A larger obliquity should correspond to a decreased annual mean tropical ocean temperature and an increased land-sea contrast in summer, and could enhance the summer monsoons, thereby decreasing the continental dust sources.

Our interpretation of the $\delta^{18}O$ and *d* GRIP data provides a consistent picture of changes occurring over the last 100,000 years in Greenland and in the North Atlantic. Our findings confirm model results by showing that

Table 1. Estimates of site temperature changes and comparison with two methods to estimate temperature changes using the water stable isotopes from GRIP ice cores. ΔT_{site} full inversion, results of the full isotopic inversion after seasonality correction; ΔT_{site} constant slope, estimate from ice $\delta^{18}O$ only, with a constant slope before 8 ka (*32*).

Period	Temperature change	Method	ΔT_{site} full inversion	ΔT_{site} constant slope
Last Glacial Maximum	–23° ± 3°C (*8*)	Borehole thermometry	–21.5° ± 3°C	–17.9°C
Bølling	11° ± 3°C (*44*)	Gas fractionation	10.9° ± 3°C	13.9°C
Younger Dryas	10° ± 4°C (*9*)	Gas fractionation	9.7° ± 3°C	11.1°C
D/O 12	12° ± 3°C (*12*)	Gas fractionation	13.9° ± 3°C	13.1°C
D/O 18	11° ± 3°C (*13*)	Gas fractionation	9.8° ± 3°C	11.4°C
D/O 19	16° ± 3°C (*11*, *13*)	Gas fractionation	17.1° ± 3°C	20.2°C
D/O 20	11° ± 3°C (*13*)	Gas fractionation	11.7° ± 3°C	16.7°C

when seasonality is accounted for, reasonable local temperature estimates can be drawn from the GRIP isotopic record; the correction due to source temperature changes is of lesser importance at glacial-interglacial scales than during rapid events. Moreover, the comparison between our estimate of ΔT_{source} and available North Atlantic sediment-based SST reconstructions suggests that large changes in geographical moisture source location occur both at the orbital and millennial scales. We show that ΔT_{source} reflects obliquity changes and that ΔT_{source} and ΔT_{site} are of opposite sign, both at orbital and millennial time scales. The influence of obliquity on deuterium excess and moisture origin, already identified for Antarctica, is confirmed for Greenland. When cold conditions prevail in the mid- and high latitudes, the moisture origin shifts to milder southward locations (*5*). Finally, we point to striking similarities between the calcium-dust records and the site-to-source temperature gradient; both are strongly modulated by obliquity, and the coupled climate model results suggest that obliquity could be linked with dust source areas through the land-sea temperature contrast. At the millennial time scale, the site-to-source temperature fluctuations highlight large-scale changes in atmospheric circulation, which is consistent with the observation of simultaneous rapid climate changes at polar, temperate, and tropical latitudes (*40*) also recorded in Greenland ice chemistry and methane fluctuations (*41*). Modeling studies have indeed shown that Northern Hemisphere storm tracks are influenced not only by the topography of the ice sheets but also by the sea-ice extent and the meridional temperature gradients (*30*).

Such large changes in the atmospheric hydrological cycle and associated climate feedbacks are not fully represented in the intermediate-complexity climate models commonly used to understand the mechanisms of abrupt events. Nonetheless, they could play a key role in the generation of instabilities of the ice sheets and the ocean circulation, amplified by changes in sea-ice extent, as has been suggested for the last glacial inception (*42*).

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Supporting Online Material
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Materials and Methods
Table S1

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A Magnetic Nanoprobe Technology for Detecting Molecular Interactions in Live Cells

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Technologies to assess the molecular targets of biomolecules in living cells are lacking. We have developed a technology called magnetism-based interaction capture (MAGIC) that identifies molecular targets on the basis of induced movement of superparamagnetic nanoparticles inside living cells. Efficient intracellular uptake of superparamagnetic nanoparticles (coated with a small molecule of interest) was mediated by a transducible fusogenic peptide. These nanoprobes captured the small molecule's labeled target protein and were translocated in a direction specified by the magnetic field. Use of MAGIC in genome-wide expression screening identified multiple protein targets of a drug. MAGIC was also used to monitor signal-dependent modification and multiple interactions of proteins.

Modern medicine faces the challenge of developing safer and more effective therapies. However, many drugs currently in use were identified without knowledge of their molecular targets (*1*, *2*). Bioactive natural products are an important source of drug leads, but their modes of action are usually unknown (*2*). Elucidation of their physiological targets is essential for understanding their therapeutic and adverse effects, thereby enabling the development of second-generation therapeutics. Moreover, the discovery of novel targets of clinically proven compounds may suggest new therapeutic applications (*3*). Target identification (ID) is also important in chemical biology, where high-throughput screening is used to identify small molecules with a desired phenotype (*4*, *5*). Despite the great benefits of such

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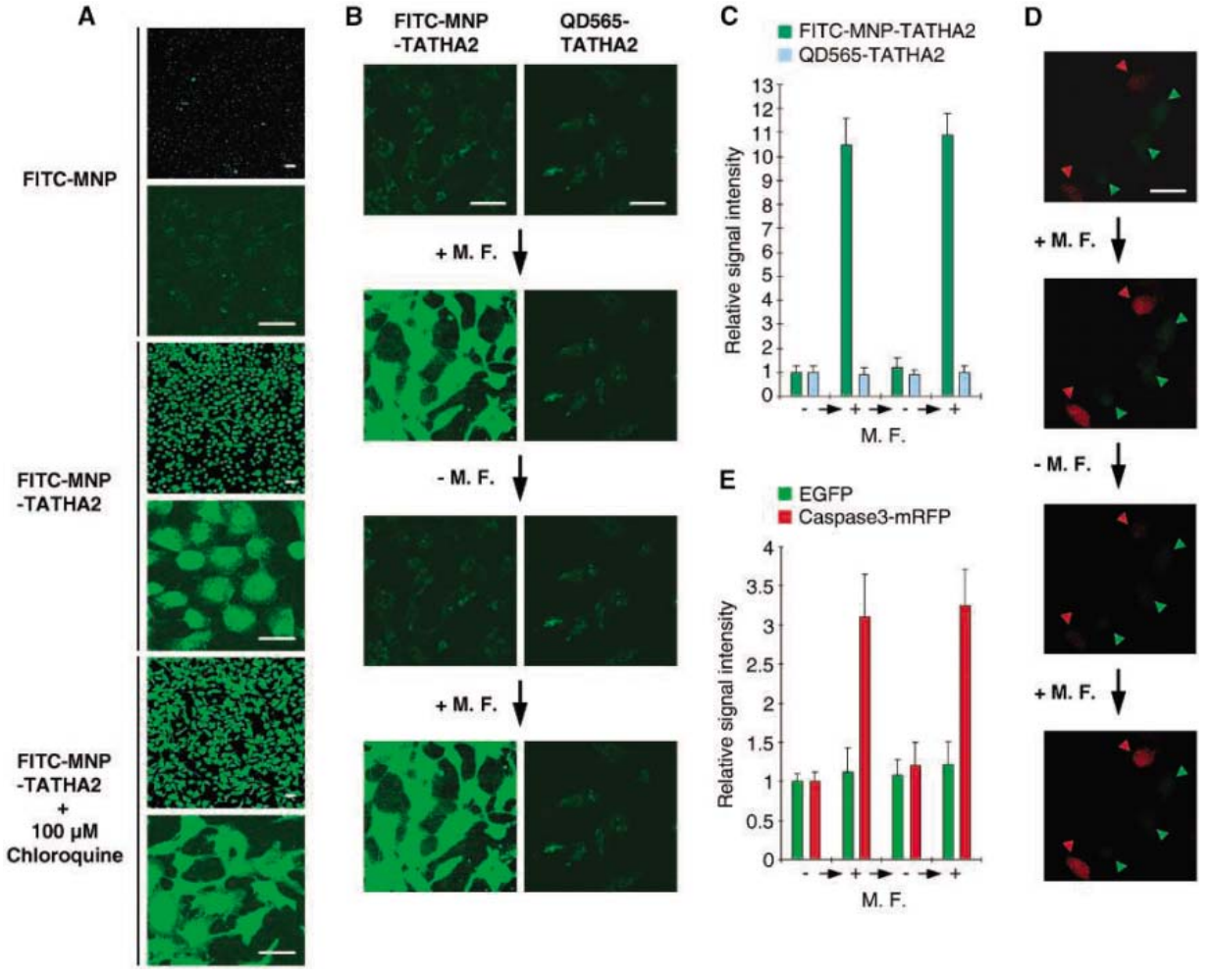

Fig. 1. Proof-of-principle experiments for MAGIC. (**A**) Enhanced intracellular uptake and endosomal escape of MNPs by TAT-HA2. HeLa cells were incubated with MNP coated with FITC (FITC-MNP) or with FITC and TAT-HA2 (FITC-MNP-TATHA2) in the absence or presence of 100 μM chloroquine for 12 hours. Live-cell confocal images were taken to show the intracellular distribution of FITC-labeled MNPs. Scale bars, 50 μm. (**B** and **C**) Translocation of MNPs inside cells by the magnetic field. HeLa cells were incubated with FITC-MNP-TATHA2 or QD565-TATHA2 in the presence of 100 μM chloroquine for 12 hours. Scale bars in (B), 50 μm. (**D** and **E**) DEVD-coated MNPs direct the translocation of caspase-3–mRFP by the magnetic field. HeLa cells were transfected with the expression plasmids for caspase-3–mRFP or EGFP, detached, mixed together, and replated. These cells were incubated with MNPs coated with DEVD and TAT-HA2 in the presence of 100 μM chloroquine for 12 hours. In (B) and (D), live-cell confocal images were taken before and after application of a magnetic field (M.F.), with the focal plane at the cellular basal surface. Cells expressing EGFP or caspase-3–mRFP are indicated by green or red arrowheads, respectively, in (D). Scale bar in (D), 50 μm. In (C) and (E), quantitative analysis of the signals was performed with more than 100 cells.

a screen, this approach has been hampered by the daunting task of target ID (*1*, *4*, *5*).

Superparamagnetic nanoparticles (MNPs) are biocompatible and are in routine clinical use (*6*, *7*). We built on these nanomagnetic probes to develop a target ID technology MAGIC, to directly probe molecular interactions inside living cells with high sensitivity and selectivity (fig. S1). Streptavidin-conjugated MNPs were used as a generic reagent to attach biotinylated molecules to the nanoprobe. After internalization of small molecule–coated MNPs into the cells, protein(s) bind to the small molecule on the MNP. Thus, when a magnetic field is applied, the MNP and associated target protein(s) can be concentrated. Fusion of a fluorescent probe to the target protein renders this translocation easily detectable by confocal microscopy.

To visualize and track the distribution of MNPs within cells, we labeled MNPs with fluorescein isothiocyanate (FITC) (*8*) (Fig. 1A). FITC-MNPs were not efficiently introduced into the cell cytosol. Most of the internalized FITC-MNPs appeared to be trapped within endocytic vesicles (Fig. 1A). Attachment of transducible fusogenic TAT-HA2 peptide on the MNPs markedly enhanced endocytic uptake and subsequent release from endosomes. It is currently understood that the high density of cationic residues in the protein transduction domain of human immunodeficiency virus TAT protein causes an electrostatic interaction with the negatively charged cell surface, thus enhancing the chance of endocytic internalization, whereas the N-terminal 20 amino acids of the influenza virus hemagglutinin protein HA2 destabilizes endosomal membranes, causing them to release their contents into the cytosol (*9*, *10*). Cotreatment with chloroquine, which is known to enhance endosome disruption (*11*), further increased the concentration of MNPs outside endosomes (Fig. 1A).

We next addressed whether internalized MNPs could be moved inside cells by an external magnetic field (Fig. 1, B and C). HeLa cells were transduced with MNPs coated with FITC and TAT-HA2. As a control, a luminescent nanocrystal quantum dot, QD565, which does not exhibit magnetism (*12*), was internalized into another set of HeLa cells with the use of TAT-HA2. Specific translocation of MNPs, but not QD565, was observed inside cells after brief application of a magnetic field (Fig. 1, B and C). Furthermore, this translocation was reversible; the

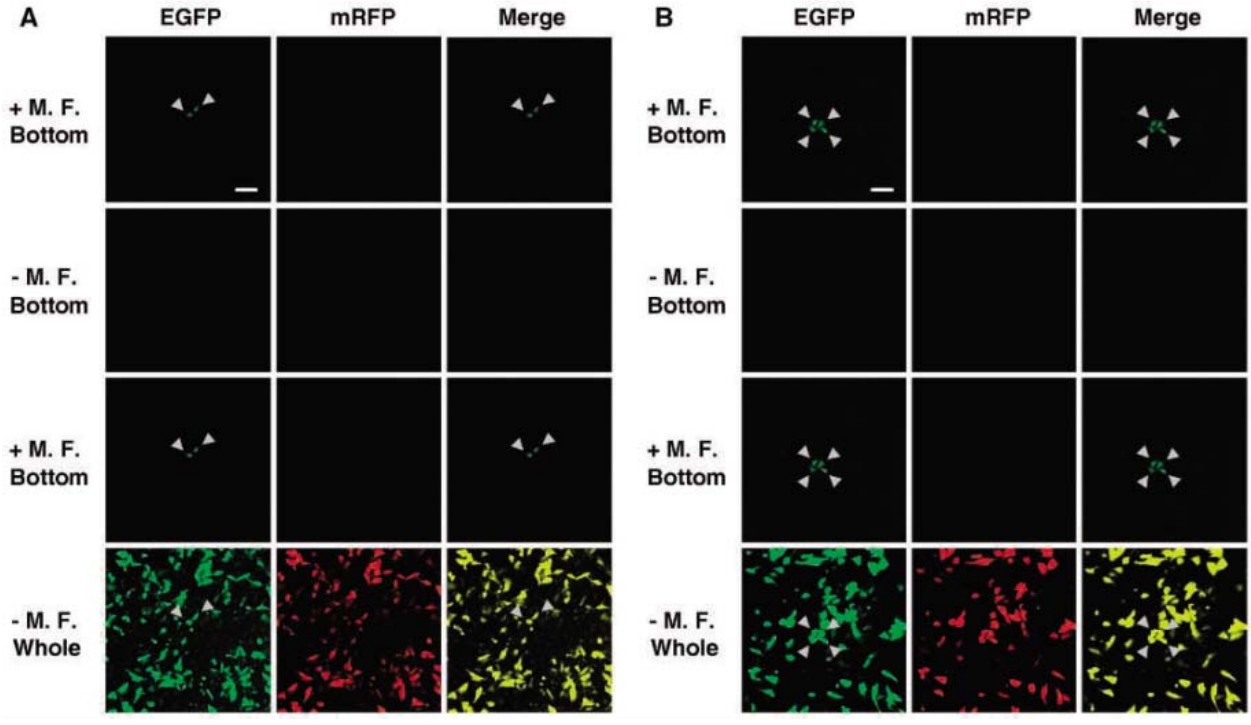

Fig. 2. Molecular target ID based on MAGIC. (**A** and **B**) HeLa cells were infected with the retroviral EGFP–fusion protein expression library (fig. S2B). After incubation of these cells with MNPs coated with FK506 and TAT-HA2 in the presence of 100 μM chloroquine for 12 hours, the subcellular localization of EGFP was examined in the absence or presence of a magnetic field (M.F.). To address potential false positives, we simultaneously monitored mRFP bicistronically coexpressed with EGFP–fusion protein. Live-cell confocal images were taken with the focal plane at the cellular basal surface (bottom) or with the pinhole size increased to collect whole-cell images (whole). Arrowheads indicate positive clones. RT-PCR and sequence analysis of mRNAs from these clones identified several proteins (Table 1) including FKBP12 (A) and an unknown protein with NCBI accession number BAB15266 (B). Scale bars, 100 μm.

Table 1. Protein targets of FK506 identified from expression cloning with the use of MAGIC. A total of 19 positive clones were identified from ~10^7 cells. The number of independent cDNA clones for each protein isolated from the screen is shown.

NCBI accession number	Protein name	Number isolated	Reported binding to FK506
NP_463460	FKBP12	4	Yes (*14–17*)
Q16645	FKBP12.6	2	Yes (*17*)
P26885	FKBP13	1	Yes (*17*)
AAA58475	FKBP25	2	Yes (*15, 17*)
Q02790	FKBP52	3	Yes (*16, 17*)
AAA86245	FKBP54	2	Yes (*17*)
NP_068758	FKBP65	1	Yes (*17*)
BAB15266	Unnamed	1	No
BAB15220	Unnamed	1	No
BAC03954	Unnamed	1	No
BAD18781	Unnamed	1	No
Total		19	

MNPs rapidly diffused away upon removal of the magnetic field and were redirected when it was reapplied.

We determined whether the MAGIC principle could be used to detect the intracellular target for Asp-Glu-Val-Asp (DEVD), an apoptosis inhibitor known to bind caspase-3 (*13*) (Fig. 1, D and E). HeLa cells were transfected with the expression construct for enhanced green fluorescent protein (EGFP) or caspase-3 fused to monomeric red fluorescent protein (mRFP). Next, these two sets of transfectants were detached, mixed, and replated for incubation with MNPs coated with DEVD and TAT-HA2. A notable amount of "red" signal, but not "green" signal, was translocated in the direction of the magnetic field (Fig. 1, D and E), indicating a specific interaction between DEVD and caspase-3 inside cells. This translocation was reversible upon removal and reapplication of the magnetic field (Fig. 1, D and E) (movie S1).

To use MAGIC in systematic target ID for a bioactive small molecule, we exploited expression cloning based on a retroviral EGFP fusion protein expression library (Fig. 2). We sought to identify the receptor(s) for an immunosuppressant, FK506 (fig. S2A), in a genome-wide screen. A normalized EGFP-tagged cDNA library was generated (*8*); cDNAs from multiple human tissues were fused to the 5′ or 3′ end of the gene encoding EGFP, and mRFP was coexpressed as an internal control to address potential false positives (fig. S2B). This library was stably expressed in HeLa cells by retroviral transduction. After introduction of FK506-coated MNPs into these cells, a magnetic field was applied, and the subcellular localization of proteins expressed from cDNA-EGFP fusions was examined. Nineteen positive clones were identified that exhibited specific translocation of EGFP in the direction of the magnetic field while the subcellular localization of mRFP remained unchanged (Table 1 and Fig. 2). Reverse transcription polymerase chain reaction (RT-PCR) and BLAST analysis of mRNAs from these clones identified overlapping transcripts of several known FK506-binding proteins and proteins of unknown function (Table 1 and Fig. 2) (*14–17*). Specific interaction of these proteins with FK506 was verified by competition analysis (fig. S3). These interactions were also readily detected with other cell types (fig. S4).

The specificity of these interactions could be distinguished readily from background and

false positive signals on the basis of reversible translocation manipulated by magnetic field at the initial screening stage (Fig. 2). Overall, the screen identified diverse targets with high efficiency and no false positives (Table 1). The additional targets discovered for FK506 might give some clues about the molecular mechanisms underlying its unexpected therapeutic actions and debilitating side effects (*17*).

To evaluate the feasibility of MAGIC for probing intracellular signaling processes, we used the NF-κB/IκB pathway (Fig. 3). Members of the NF-κB family (e.g., RelA/p65) are sequestered in the cytoplasm by IκB family members (e.g., IκBα). Various stimuli, including proinflammatory cytokines such as tumor necrosis factor–α (TNF-α), induce the phosphorylation of IκBα on two serine residues, Ser^{32} and Ser^{36} (*18*). This phosphorylation results in recognition of IκBα by the F box protein βTrCP of the SKP1/cullin/F-box ubiquitin ligase complex, leading to ubiquitin-dependent proteolysis of IκBα. These regulated protein-protein interactions allow NF-κB proteins to translocate into the nucleus, resulting in the expression of target genes.

We first tested whether signal-induced protein phosphorylation could be detected with MAGIC (Fig. 3, A to D). After expression of IκBα fused to mRFP in HeLa cells, these cells were loaded with MNPs triple-labeled with TAT-HA2, FITC, and antibody to phosphorylated Ser^{32} of IκBα (*8*). Before stimulation, translocation of IκBα-mRFP was barely detectable, despite active accumulation of FITC signal in the direction of the magnetic field (Fig. 3, B to D). In contrast, brief stimulation with TNF-α markedly induced the magnetic field–directed translocation of mRFP signal for IκBα, faithfully reflecting the phosphorylation of IκBα in response to TNF-α inside cells (fig. S5). Under these conditions, SC-514, an inhibitor of IκB kinase (IKK), prevented TNF-α–induced translocation of IκBα-mRFP (Fig. 3, C and D), further emphasizing the specificity of the method.

Next we examined whether signal-dependent protein-protein interactions could be detected with MAGIC (Fig. 3, A, E to G). To entrap IκBα with MNPs inside cells, we used MNPs coated with FK506 and TAT-HA2 (TATHA2-MNP-FK506) together with IκBα tagged with FK506-binding protein, FKBP12 (IκBα-ECFP-FKBP12; Fig. 3, A, E to G). After expression of IκBα-ECFP-FKBP12, EYFP-RelA, and mRFP-βTrCP in HeLa cells, these cells were transduced with TATHA2-MNP-FK506. Application of a magnetic field directed the translocation of ECFP signal (Fig. 3F), reflecting IκBα recruitment around MNPs by FK506-FKBP12 interaction inside cells. Under these conditions, EYFP-RelA, but not mRFP-βTrCP, was directed toward the magnetic field, indicating that interaction occurs between IκBα and RelA, but not between IκBα and βTrCP. Upon stimulation with TNF-α, a marked increase in mRFP-βTrCP translocation was observed (Fig. 3, F and G), demonstrating signal-induced interaction between IκBα and βTrCP. Notably, SC-514 blocked TNF-α–induced translocation of mRFP-βTrCP (Fig. 3, F and G). Thus, MAGIC can faithfully probe molecular interactions dynamically regulated by specific signals in live cells.

In certain disease conditions (e.g., systemic inflammation), several cellular functions, such as endocytosis, are known to be compromised (*19*, *20*). To demonstrate MAGIC in cells impaired in endocytosis, we adopted microinjection that can introduce exogenous materials directly into cells without passing through endocytic vesicles (*21*) (fig. S6). The signal-induced phosphorylation and protein-protein

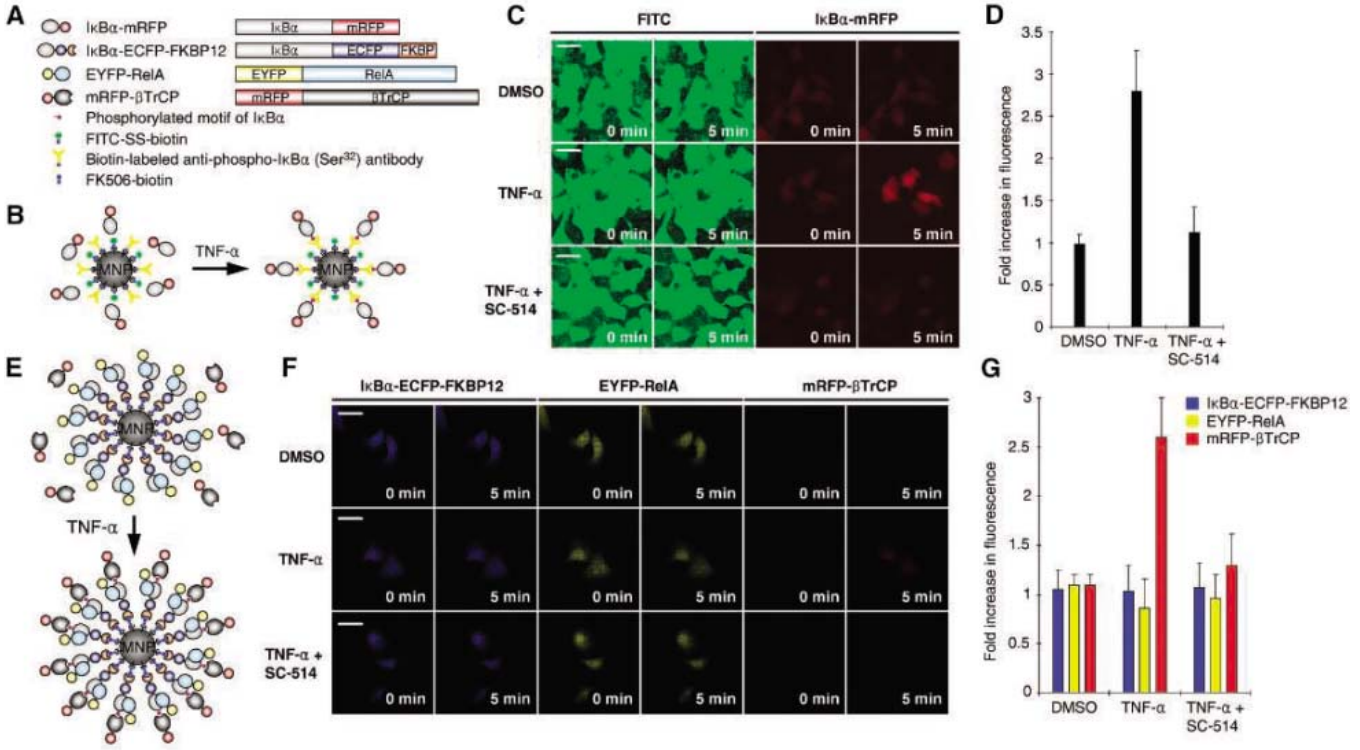

Fig. 3. Monitoring biological signaling processes by MAGIC. (**A**) Schematic of IκBα-mRFP, IκBα-ECFP-FKBP12, EYFP-RelA, and mRFP-βTrCP. Symbols used in (B) and (E) are also explained. (**B** to **D**) Detection of signal-induced phosphorylation of IκBα. After transfection of HeLa cells with the expression plasmid for IκBα-mRFP, MNPs coated with antibody to phosphorylated Ser^{32} of IκBα, FITC, and TAT-HA2 were introduced into these cells. Magnetic field–induced translocation of FITC and mRFP signals was monitored before and after stimulation with TNF-α (10 ng/ml) for 5 min in the absence or presence of prior treatment with SC-514 (1 mM). (**E** to **G**) Detection of signal-dependent association of IκBα with βTrCP. After transfection of HeLa cells with the expression plasmids for IκBα-ECFP-FKBP12, EYFP-RelA, and mRFP-βTrCP, MNPs coated with FK506 and TAT-HA2 were introduced into these cells. Magnetic field–induced translocation of enhanced cyan fluorescent protein (ECFP), enhanced yellow fluorescent protein (EYFP), and mRFP signals was monitored before and after stimulation with TNF-α (10 ng/ml) for 5 min in the absence or presence of prior treatment of SC-514 (1 mM). Scale bars, 50 μm. Shown in (D) and (G) are quantitative analyses of induced signals after stimulation with TNF-α for 5 min with more than 100 cells.

interaction between IκBα and RelA were probed in HeLa cells pretreated with TNF-α, which inhibited the endocytic uptake of MNPs, as described (*20*). MNPs coated with antibody to phosphorylated Ser^{32} of IκBα or with FK506 were microinjected into TNF-α–pretreated HeLa cells expressing IκBα-mRFP or IκBα-ECFP-FKBP12/EYFP-RelA/mRFP-βTrCP, respectively (*8*). Signal-induced phosphorylation and interaction of the NF-κB/IκB pathway were readily detected by MAGIC in this experimental setting (fig. S6).

MAGIC offers several advantages over target ID methods currently in use. First, it directly translates a physical molecular interaction into a clear readout signal, unlike indirect readout methods that are dependent on intermediary interactions (*22*), overall expression profiles (*23*), or complex biological phenotypes (*24*). Thus, intrinsic false positives/negatives or error-prone deductions about molecular target(s) of a small molecule are obviated. Second, by probing such interactions in a physiologically relevant context, misleading outcomes produced by an artificial experimental setting (*22*, *24*–*28*) can be greatly diminished. Third, it is amenable to dynamic, single-cell analysis of interactions. Finally, MAGIC can be used to detect a variety of biological interactions and protein modifications within live cells in a broad range of tissues and disease states. With the great advantage of being able to detect dynamic interactions between the biomolecules within mammalian cells, this technology could be exploited in genome-wide interaction screens.

The benefits of MAGIC may be best achieved through efficient and nondisruptive introduction of MNPs into cells. Prolonged incubation of cells with TAT-HA2–conjugated MNPs may affect cellular physiology, and microinjection cannot be used for large populations of cells. Other technologies for delivering biologically active cargos into cells (*10*) will be helpful to complement MAGIC.

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Cell-to-Cell Transfer of Bacterial Outer Membrane Lipoproteins

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Myxococcus xanthus **cells can glide forward by retracting type IV pili. Tgl, an outer membrane lipoprotein, is necessary to assemble pili. Tgl mutants can be transiently "stimulated" if brought into end-to-end contact with *tgl*⁺ donor cells. By separating the stimulated recipient cells from donor cells, we found that Tgl protein was transferred from the donors to the rescued recipient cells. Mutants lacking CglB lipoprotein, which is part of a second gliding engine, could also be stimulated, and CglB protein was transferred from donor to recipient cells. The high transfer efficiency of Tgl and CglB proteins suggests that donor and recipient cells briefly fuse their outer membranes.**

Myxobacteria move by gliding on surfaces—an effective mode of translocation in their soil habitat (*1*). *Myxococcus xanthus* moves smoothly in the direction of its long axis, then reverses and moves in the opposite direction. It has no flagella and cannot swim; its gliding is propelled by two polar engines that are encoded by the A and the S genes (*2*). The A engines appear to extrude polymer chains from the trailing ends of cells, which gelate, pushing the cells forward (*3*). Slime is extruded from the trailing end only (*4*). The S genes encode hairlike appendages projecting from the leading end of the cell—the type IV pili (*5*, *6*)—and they pull a cell forward by retracting (*7*). Many proteobacterial pathogens have type IV pili (*8*). *M. xanthus* type IV pili are polar (*6*), and S motility is limited to cells within pilus-contact distance of each other (*9*).

A common set of 10 proteins is necessary for the assembly and retraction of type IV pili in *M. xanthus*, *Pseudomonas aeruginosa*, and *Neisseria gonorrhoeae* (*8*). One of those proteins, known as PilA in *M. xanthus*, constitutes the pilus filament, a helical array of PilA monomers (*10*). The filament is thought to pass through the outer membrane within a gated channel formed by the PilQ secretin protein (*11*). Secretins are multimeric channels large enough to pass folded proteins (*12*). Secretins often have cognate lipoproteins that facilitate their assembly in the outer membrane (*13*, *14*).

The Tgl lipoprotein is necessary to assemble a detergent-resistant PilQ secretin in *M. xanthus* (*15*). Nevertheless, *tgl* mutants can be stimulated to assemble pili and to glide after transient contact with *tgl*⁺ donor cells (*16*, *17*). Cells with a *tgl* mutation lack S motility but have normal A motility. Tgl stimulation specifically restores their S motility (*2*). To render stimulation visible by its effect on motility, we transferred a mutant *tgl* locus into an A⁻ background, creating a nonmotile strain. Strain DK8602 (table S1) lacked both A and S motility and formed a smooth-edged colony (Fig. 1A). When DK8602 was mixed with a nonmotile *tgl*⁺ donor strain (Δ*pilA*, *aglB1*), the mixed population initially had a smooth colony edge reflecting both strains' lack of A and S motility (Fig. 1A). Recipient cells swarm beyond the original colony edge as flares when they are stimulated (Fig. 1B), which shows that the cells had assembled retractile pili.

To distinguish donor from recipient cells in a Tgl-stimulation mixture, we expressed the green fluorescent protein (GFP) in one population or the other. When the recipient strain

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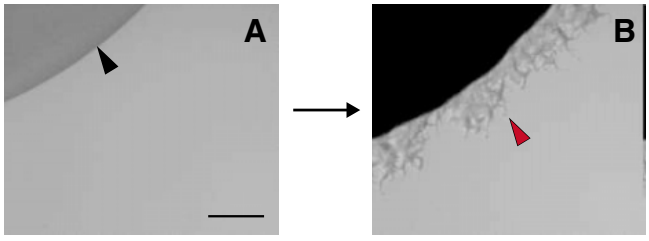

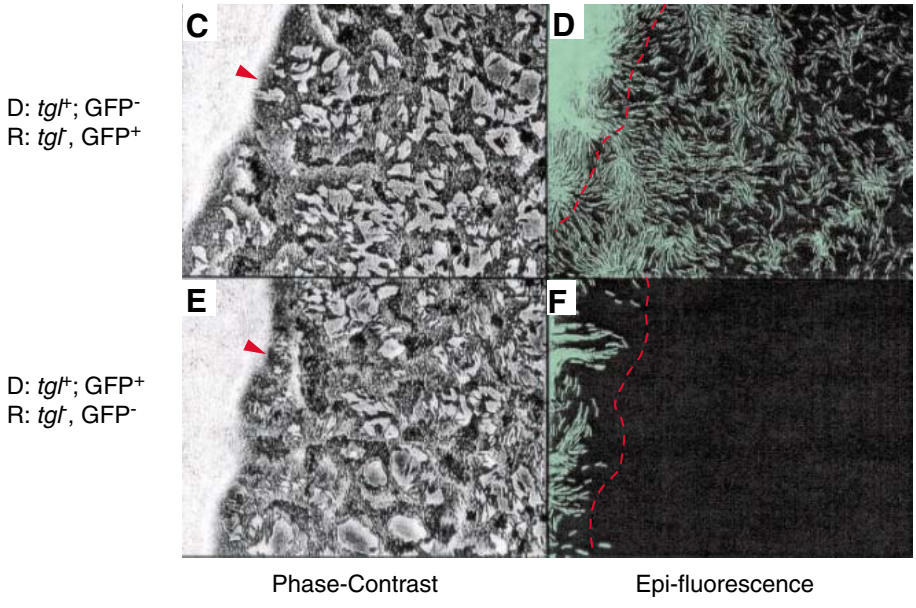

Fig. 1. Tgl stimulation. Stimulated recipient cells became motile and swarmed outward, the donor strain remained nonmotile; it was found inside the original edge of the colony. A *tgl* mutant carrying an A^- mutation (A^-S^- strain DK8602) was mixed with another nonmotile tgl^+ mutant strain (DK8601) and spotted on agar. **(A)** At 0 hours, the mixed colony had a smooth edge (black arrowhead) because there were no motile cells (Scale bar, 500 μm). **(B)** After several hours, the tgl^+ donor cells activate S motility in the *tgl* mutant recipient cells by stimulation. The outward swarming of the stimulated recipients after 4 days is indicated by the red arrowhead. This motility was transient; it lasted only a week (fig. S2). **(C)** Phase-contrast image of DK8601 (GFP^- donor cells) mixed 1:1 with a mixture of DK8607 [GFP^+ recipient cells (table S1)] and DK8602 (GFP^- recipient cells), the two last-named strains at a 1:50 ratio. The original colony edge is indicated by the red arrowhead. **(D)** Epifluorescent image of the field shown in (C). The original colony edge is indicated by the dashed red line. **(E)** Phase-contrast image of DK8602 (GFP^- recipient cells) mixed 1:1 with a mixture of DK8606 (GFP^+ donor cells) and DK8601 (GFP^- donor cells) at a 1:50 ratio. **(F)** Epifluorescent image of (E).

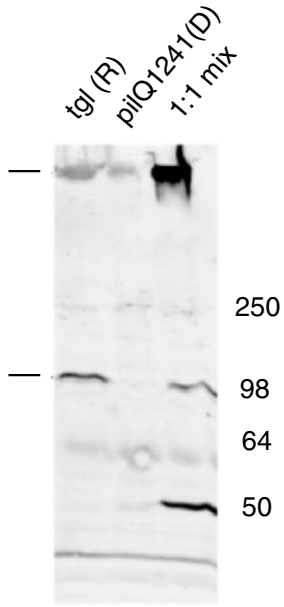

Fig. 2. Stimulated assembly of PilQ multimers. A mutant strain with an in-frame deletion in *tgl* failed to assemble PilQ multimers (top line to left of gel), although it produced the 98-kD PilQ monomer (other line on left). When the Δ*tgl* strain was stimulated with the donor strain tgl^+ $pilQ^{1241}$(a pilQ allele that produces unstable PilQ monomers in lane 2), boiling SDS–resistant PilQ multimers apparently formed at the expense of monomers (lane 3).

DK8607 (Δ*tgl*, *aglB1*) contained GFP, it was seen to move out in characteristically S-motile flares (Fig. 1, C and D). By contrast, when GFP was expressed in the donor strain DK8606 (Δ*pilA*, *aglB1*), it was not found beyond the original colony edge (Fig. 1F). Because only stimulated recipients swarmed out, it was possible to harvest pure cultures of stimulated cells. The genotype of such harvested cells was tested by colony polymerase chain reaction (PCR) that specifically amplified the *tgl* gene. As expected, pure cultures of donor cells had a *tgl* gene, and pure cultures of Δ*tgl* recipient cells lacked it (fig. S1). Furthermore, harvested stimulated recipient cells lacked the *tgl* gene (fig. S1).

Because Tgl is required for the assembly of (detergent-resistant) PilQ multimers (*15*), the S motility of stimulated cells implies that those cells have assembled PilQ multimers, because they are able to extend and retract pili. To investigate the assembly of PilQ in stimulated recipients, a donor strain was needed that lacked such assemblies. The Δ*pilQ* tgl^+ strain is not an efficient donor for Tgl stimulation (*15*). However, although a $pilQ^{1241}$ nonsense mutant did not assemble stable PilQ multimers (*15*), it could nevertheless serve as a Tgl donor. When a tgl^+ $pilQ^{1241}$ donor strain was mixed with *tgl* recipients, assembled PilQ multimers were readily detected by their resistance to dissociation after being boiled in SDS-containing buffer (Fig. 2). Unassembled PilQ monomers were also found in the stimulated recipients, as well as a 50-kD PilQ fragment that always accompanied PilQ assembly (*15*). The recipients before stimulation had only unassembled PilQ monomers and no 50-kD fragment (Fig. 2). The donor did not have assembled PilQ multimers, 50-kD fragment, or $PilQ^{1241}$ monomers (Fig. 2). It appears that the mutant monomers whose carboxy ends had been truncated were degraded.

We tested the possibility that Tgl protein was transferred from donor to recipient cells. As expected, Tgl protein was found in the donor cells, but not in the recipient cells (Fig. 3A). However, after stimulation, large amounts of Tgl protein were also found in the harvested stimulated recipient cells (Fig. 3A). Thus, transient contact between Tgl^+ donor cells and Tgl^- recipients results in the transfer of Tgl protein from donor to recipient. To challenge the specificity of transfer, we examined a cytoplasmic protein, GFP, in donor cells, recipient cells, and stimulated recipient cells. When only recipients contained GFP, it was detected only in the recipient cells and the harvested stimulated recipient cells (Fig. 3A). Conversely, when only donors contained GFP, it was detected only in the donor cells (Fig. 3A), which demonstrated the absence of contaminating donor cells in the harvested stimulated recipient cells. This experiment also shows that GFP is not transferred.

Transfer of Tgl protein was also demonstrated in cells separated by flow cytometry. The (GFP^-) donor strain DK8601 was mixed 1:1 with the fluorescent GFP^+ recipient strain DK8607, and the mixture was spotted on agar.

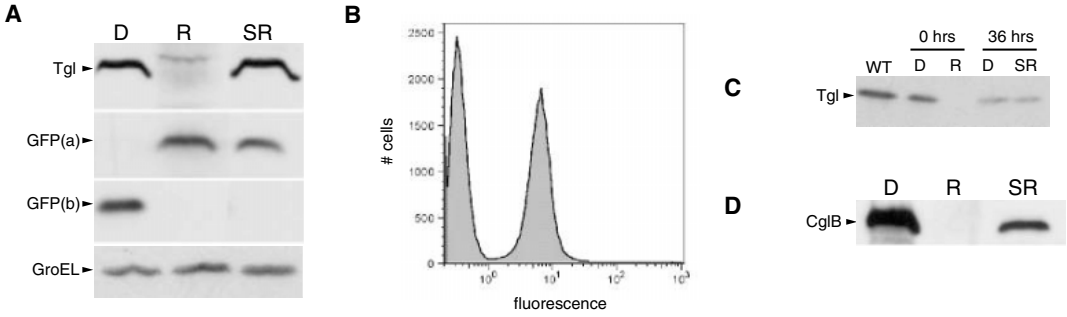

Fig. 3. Detection of Tgl protein transfer from donor cells to recipient cells. (**A**) Immunoblots lane 1, donor cells, DK8606; lane 2, recipient cells, DK8602; and lane 3, the harvested stimulated recipient cells (SRs). The GFP(a) row is a fluorogram of GFP$^-$ donor strain, DK8601, GFP$^+$ recipient, DK8607, and their harvested SRs. The GFP(b) row shows GFP$^+$ donor, DK8606, the GFP$^-$ recipient, DK8602, and their harvested SRs. GroEL is the loading control. (**B**) Fluorescence profile of a mixture of GFP$^-$ donor cells (strain DK8601) with GFP$^+$ recipient cells (strain DK8607), separated by fluorescence-activated cell sorting (FACS) 36 hours after mixing. (**C**) Tgl Immunoblot of fractions from the FACS separation in (B), 0 hours after mixing and 36 hours after mixing. (**D**) Transfer of the CglB protein from donor (strain DK6204) to recipient cells (strain ASX1). The two peaks evident in B were harvested separately.

After 36 hours, the cells remained in a 1:1 ratio in two distinct populations: GFP$^-$, which has 0.35 units of autofluorescence, and GFP$^+$, which has 8 units of GFP fluorescence (Fig. 3B). The presence of the Tgl protein was evaluated before and after stimulation. As expected, before stimulation, Tgl was present in the donor cells and absent in the recipients (Fig. 3C). After 36 hours of mixed swarming, Tgl was detected in both the donor cells and the population of stimulated recipient cells (Fig. 3C).

The only gene that can be stimulated in the S motility system is Tgl. However, five A motility genes can be stimulated: *cglB*, *cglC*, *cglD*, *cglE*, and *cglF* (*17*). The *cglB* gene has been cloned and sequenced; it encodes a lipoprotein that has no similarity to *tgl*, apart from a type II signal sequence (*18*). To test protein transfer in A motility stimulation, stimulated *cglB* recipient cells were separated from the donor cells after they had spread beyond the edge of the original spot. Indeed, the harvested, stimulated *cglB* mutant recipients contained large amounts of the CglB protein (Fig. 3D).

The concentration of Tgl and CglB proteins in stimulated cells was similar to the concentration in donor cells (Fig. 3), as if the donor and recipient cells shared their outer membrane lipoproteins equally. Myxobacteria may have evolved an efficient sharing of outer membrane lipoproteins, because they need to reverse their gliding direction frequently, 20 or more times per division cycle (*19*). Frequent reversal means frequently reconstructing the A and the S engines (*4*). Tgl and CglB (and perhaps CglC, D, E, and F) are needed to specify which cell poles have pili and which are active in slime secretion. This sharing of outer membrane lipoproteins among the thousands of cells in a swarm creates a primitive tissue.

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Ubiquitination on Nonlysine Residues by a Viral E3 Ubiquitin Ligase

Ken Cadwell and Laurent Coscoy*

Ubiquitination controls a broad range of cellular functions. The last step of the ubiquitination pathway is regulated by enzyme type 3 (E3) ubiquitin ligases. E3 enzymes are responsible for substrate specificity and catalyze the formation of an isopeptide bond between a lysine residue of the substrate (or the N terminus of the substrate) and ubiquitin. MIR1 and MIR2 are two E3 ubiquitin ligases encoded by Kaposi's sarcoma–associated herpesvirus that mediate the ubiquitination of major histocompatibility complex class I (MHC I) molecules and subsequent internalization. Here, we found that MIR1, but not MIR2, promoted down-regulation of MHC I molecules lacking lysine residues in their intracytoplasmic domain. In the presence of MIR1, these MHC I molecules were ubiquitinated, and their association with ubiquitin was sensitive to β_2-mercaptoethanol, unlike lysine-ubiquitin bonds. This form of ubiquitination required a cysteine residue in the intracytoplasmic tail of MHC I molecules. An MHC I molecule containing a single cysteine residue in an artificial glycine and alanine intracytoplasmic domain was endocytosed and degraded in the presence of MIR1. Thus, ubiquitination can occur on proteins lacking accessible lysines or an accessible N terminus.

Ubiquitination is a highly regulated process conserved in all eukaryotes (*1*, *2*) that regulates many fundamental cellular processes. Many pathogens mimic, block, or redirect the activity of the ubiquitin system. The modulators of immune recognition (MIR) 1 and 2, two proteins encoded by Kaposi's sarcoma–associated herpesvirus (KSHV), specifically down-regulate the expression of MHC I from the surface of infected cells, presumably to prevent lysis of infected cells by cytotoxic T lymphocytes (*3*–*6*). MIR1 and MIR2 are highly homologous structurally and functionally, and they belong to a large family of E3 ubiquitin

ligases (*3*, *7*). E3 ubiquitin ligases function as adaptors to facilitate positioning and transfer of ubiquitin (Ub) from an E2 enzyme directly onto the E3-bound substrate (*1*). The nature of the bond between Ub and its substrate has been well characterized: The Ub C-terminal glycine carboxy group forms an isopeptide bond with the ε-amino group of lysine residues or, less commonly, with the amino group at the N terminus of the substrate protein (*8*). MIR proteins recruit E2 enzymes with their N-terminal RING-CH domain (*3*). Either direct or indirect interactions between the transmembranes of the MIRs and MHC I molecules ultimately lead to the ubiquitination of lysine residues present in the MHC I intracytoplasmic tail (*3*, *9*). Ubiquitinated molecules are then endocytosed and degraded by the lysosome (*3*, *7*, *10*–*12*). Mutating all the lysines to arginines in the intracytoplasmic domain of HLA.B7 (henceforth referred to as the HLA.B7 2R mutant or lysineless HLA.B7) abolishes internalization mediated by MIR2 (*3*).

However, in the presence of MIR1, the cell surface expression of both wild-type (wt) HLA.B7 and HLA.B7 2R was strongly down-regulated, even in cells expressing low levels of MIR1 (Fig. 1). In contrast, even high levels of MIR2 (Fig. 1) did not induce HLA.B7 2R down-regulation. Thus, the MIR1 protein can mediate the down-regulation of MHC I molecules lacking lysines. Similar results were observed in HeLa cells, suggesting that HLA.B7 2R down-regulation by MIR1 is not restricted to B cells. In the presence of MIR1, HLA.B7 2R molecules are endocytosed, translocated toward the lysosome, and degraded, which is similar to the effects of MIR1 on HLA.B7 wt molecules.

To test whether a particular motif encoded in the intracytoplasmic domain of HLA.B7 2R was required for MIR1-mediated down-regulation, we generated a set of HLA.B7 2R molecules lacking different parts of the intracytoplasmic domain (Fig. 2A) and tested their susceptibility to MIR1-mediated down-regulation. Deletion of the last seven amino acids in HLA.B7 2R did not prevent down-regulation (HLA.B7 ΔC), whereas further truncations (constructs HLA.B7 Δ, ΔA, and ΔB) inhibited internalization (Fig. 2C). Thus, a critical determinant for MIR1-mediated down-regulation is encoded in the last seven residues of HLA.B7 ΔC.

Although we observed down-regulation of lysineless HLA.B7 by MIR1, a lysine-less HLA.A2 molecule is not down-regulated by MIR1 (*7*). Within the region identified above, HLA.B7 encodes a cysteine in the same position that HLA.A2 encodes a serine (Fig. 2B). We generated a HLA.B7 mutant lacking both cysteine and lysine in its cytoplasmic tail, which we call HLA.B7 2RS (Fig. 2B). Mutation of the cysteine decreased the extent of MIR1-mediated endocytosis (Fig. 2D). We then introduced by mutagenesis a cysteine into the intracytoplasmic tail of HLA.A2 in which all the intracytoplasmic lysines were previously mutated (Fig. 2B). HLA.A2 without lysines (HLA A2 3R) was slightly down-regulated in the presence of MIR1, and introduction of the cysteine in HLA.A2 3R (HLA.A2 3RC) allowed full down-regulation (Fig. 2E). We also substituted the last arginine residue of HLA.B7 2RS (HLA.B7 without lysines or cysteines) with a cysteine and observed that this mutant was as susceptible as HLA.B7 wt to MIR1-mediated down-regulation (Fig. 2, B and D). This strongly suggested that the cysteine was not acting within a linear motif. Thus, in addition to the lysine- and Ub-dependent pathway, MIR1 can down-regulate surface molecules in a lysine-independent manner through a process that requires a cysteine in the intracytoplasmic tail of the target molecule. Other determinants may also be important, because HLA.B7 2RS and HLA.A2 3R, neither of which contains lysines or cysteines, are both partially down-regulated (Fig. 2, D and E).

To further demonstrate that a single cysteine was sufficient to promote MIR1-mediated down-regulation, we replaced the intracytoplasmic tail of HLA.B7 by a stretch of glycine and alanine residues (GA stretch) (Fig. 3A). To this GA stretch, we added each of the 20 amino acids at position X (Fig. 3B). As expected, the GA stretch did not allow down-regulation, whereas the presence of a lysine did. Thus, lysine is sufficient to promote ubiquitination-mediated down-regulation independent of surrounding motifs. The same phenotype was observed in the presence of a cysteine (Fig. 3B). None of the other amino acids lead to down-regulation in the presence of MIR1 (Fig. 3B), except serine. The extent of the down-regulation in the presence of a serine was modest but highly reproducible. This is consistent with the fact that HLA.B7 2R (which does not have lysines but has a cysteine) is strongly down-regulated, and HLA.B7 2RS (no lysines or cysteine) is partially down-regulated (Fig. 2D). Indeed, HLA.B7 2RS contains nine serine residues in its cytoplasmic tail. Overall, it appears

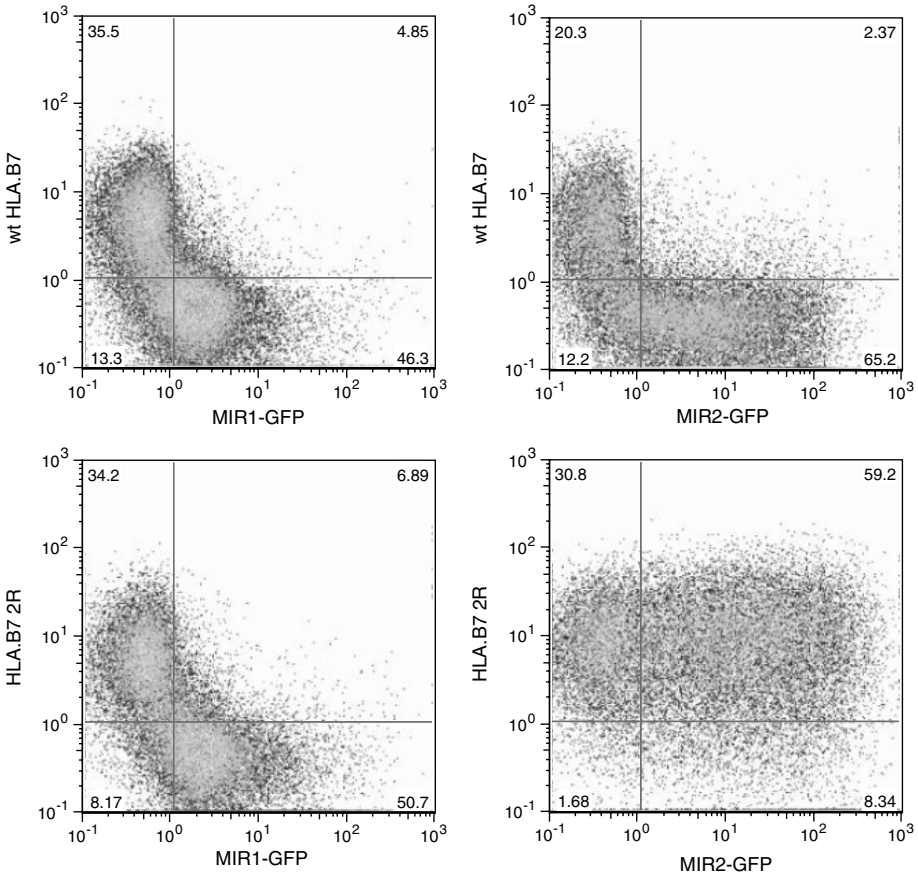

Fig. 1. MIR1, but not MIR2, down-regulates the MHC I allele HLA.B7 in the absence of intracytoplasmic lysines. BJAB cells stably expressing wt HLA.B7 or the HLA.B7 2R mutant lacking the two intracytoplasmic lysines were transiently transfected with a vector expressing MIR1 or MIR2 fused to enhanced green fluorescent protein (EGFP). Cells were stained with a phycoerythrin-conjugated monoclonal antibody against HLA.B7 and analyzed by flow cytometry.

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that MHC I molecules can be down-regulated independently of lysines, in a cysteine-dependent (and possibly serine-dependent) fashion.

We examined the possibility that lysineless MHC I molecules could be ubiquitinated in the presence of MIR1. We used the hamster CHO cell line, which is permissive for MIR1-mediated down-regulation and does not express endogenous human MHC I molecules. We stably transduced CHO cells with the different HLA.B7 constructs and MIR1. After selection, human MHC I heavy chains were specifically immunoprecipitated, and their ubiquitination status was analyzed. No ubiquitinated forms were observed in the absence of MIR1, or when MIR1 was coexpressed with the HLA.B7 construct lacking almost all its intracytoplasmic domain, HLA.B7 Δ (Fig. 4A). However, in cells expressing MIR1, ubiquitination of HLA.B7 wt and, to a lesser extent, HLA.B7 2R (no lysines) was readily detectable, because it produced a characteristic heterogeneous array. In addition, a small but detectable degree of ubiquitination was observed in HLA.B7 2RS (no lysines or cysteine), consistent with the lower level of down-regulation observed (Fig. 4B). Thus, a residue other than lysine was being ubiquitinated by MIR1.

We next examined the possibility that this cysteine was the ubiquitination site for HLA.B7 2R. We immunoprecipitated wt HLA.B7, as well as HLA.B7 2R, from CHO cells expressing MIR1, and we incubated these immunoprecipitates in the presence of β_2-mercaptoethanol at pH 11 in order to break potential thiol-ester bonds (cysteine-Ub bond) but not isopeptide bonds (lysine-Ub bond). Ubiquitination of HLA.B7 2R, but not HLA.B7 wt, was completely eliminated by this treatment (Fig. 4, B and C). Similarly, treatment drastically diminished the cysteine-Ub bond from the E2 enzyme UBE2E3 (fig. S1).

Altogether, our results show that in the absence of lysine, HLA.B7 molecules are ubiquitinated in a cysteine-dependent manner. Moreover, the bond between ubiquitin and the lysineless HLA.B7 shares the same chemical property as the bond between ubiquitin and E2s, which strongly suggests that cysteine is the ubiquitin-attachment site for HLA.B7 2R. Direct visualization of the cysteine-ubiquitin bond by mass spectrometry is hindered by the small amount of ubiquitinated molecules available for purification.

The foregoing shows that the side chain of residues other than lysine can serve as receptors for substrate ubiquitination. It is puzzling that, although ubiquitination has been extensively studied, in particular using large-scale proteomic, such a modification has never been observed in the past. A thiol-ester bond (cysteine-ubiquitin) is more labile than an isopeptide bond (lysine-ubiquitin), which certainly hinders its detection. This may explain why the level of ubiquitination detected with HLA.B7 2R is not as robust as the one observed with HLA.B7 wt (Fig. 4A). In addition, we believe that this form of ubiquitination might be restricted to a subfamily of E3 ubiquitin ligases, such as the MIR1 E3 ubiquitin ligase family (MIR1 and its homologs) (*13*) (SOM text and fig. S2). The regulation processes mediated by ubiquitination may be more complex because nonlysine residues are also targets of ubiquitination. For example, the number of potential substrates could be extended to molecules that do not contain accessible lysines or an accessible N terminus, and/or transient ubiquitination of substrates may occur, because thiol-ester bonds (Ub-cysteine) are more labile than isopeptide bonds (Ub-lysine). It will be important to determine whether this alternate form of ubiquitination requires the same cellular cofactors as the ones involved in lysine ubiqui-

A

	Transmembrane / Intracytoplasmic Tail
HLA.B7 Δ	----VVIGAVVAAVMCR
HLA.B7 ΔA	----VVIGAVVAAVMCRRRSSGGRG
HLA.B7 ΔB	----VVIGAVVAAVMCRRRSSGGRGGSYSQAA
HLA.B7 ΔC	----VVIGAVVAAVMCRRRSSGGRGGSYSQAA**CSDSAQG**
HLA.B7 2R	----VVIGAVVAAVMCRRRSSGGRGGSYSQAACSDSAQGSDVSLTA

B

	Transmembrane / Intracytoplasmic Tail
wt HLA.B7	----VVIGAVVAAVMCRR **K**SSGG**K**GGSYSQAA**C**SDSAQGSDVSLTA
HLA.B7 2R	----VVIGAVVAAVMCRR**R**SSGG**R**GGSYSQAA**C**SDSAQGSDVSLTA
HLA.B7 2RS	----VVIGAVVAAVMCRR**R**SSGG**R**GGSYSQAA**S**SDSAQGSDVSLTA
HLA.B7 RC	----VVIGAVVAAVMCRR**R**SSGG**C**GGSYSQAA**S**SDSAQGSDVSLTA
wt HLA.A2	----VITGAVVAAVMWRR**K**SSDR**K**GGSYSQAA**S**SDSAQGSDVSLTACKV
HLA.A2 3R	----VITGAVVAAVMWRR**R**SSDR**R**GGSYSQAA**S**SDSAQGSDVSLTAC**R**V
HLA.A2 3RC	----VITGAVVAAVMWRR**R**SSDR**R**GGSYSQAA**C**SDSAQGSDVSLTAC**R**V

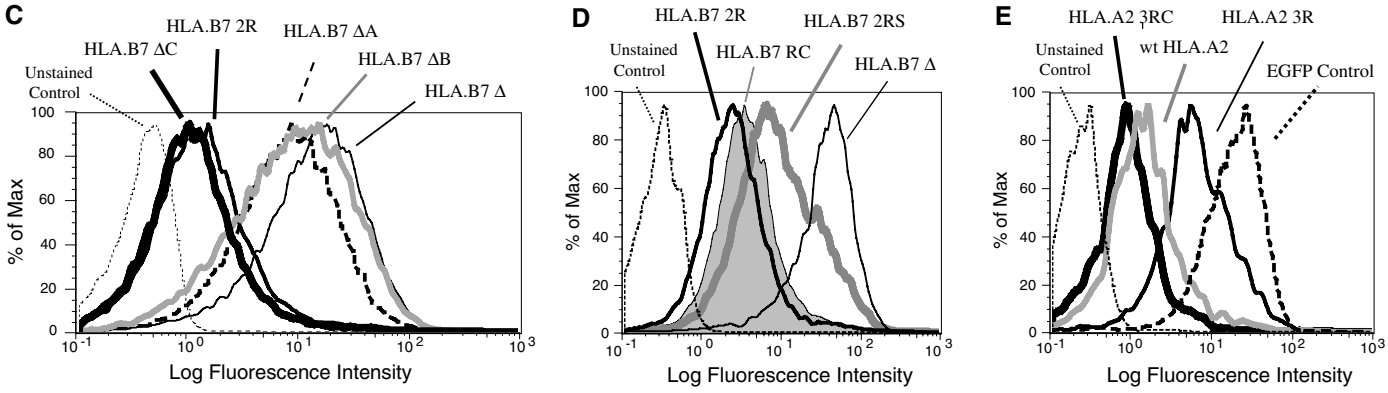

Fig. 2. An intracytoplasmic cysteine residue is critical for lysine-independent down-regulation by MIR1. (**A**) Polymerase chain reaction (PCR) mutagenesis was used to create serial deletion mutants in the intracytoplasmic tail of HLA.B7 2R. (**B**) We generated several mutations within the intracytoplasmic region of HLA.B7 and HLA.A2 so as to analyze the requirement of the cysteine residue unique to HLA.B7. (**C**) BJAB cells stably expressing the mutants in (A) were transiently transfected with a construct expressing MIR1-EGFP and analyzed for surface expression of HLA.B7 by flow cytometry. (**D**) BJAB cells stably expressing MIR1-EGFP along with the various HLA.B7 mutants were analyzed for surface HLA.B7 expression by flow cytometry. (**E**) HeLa cells stably expressing MIR1-EGFP and various HLA.A2 mutants were analyzed for surface HLA.A2 expression. No down-regulation is indicated by cells stably expressing HLA.A2 and EGFP alone.

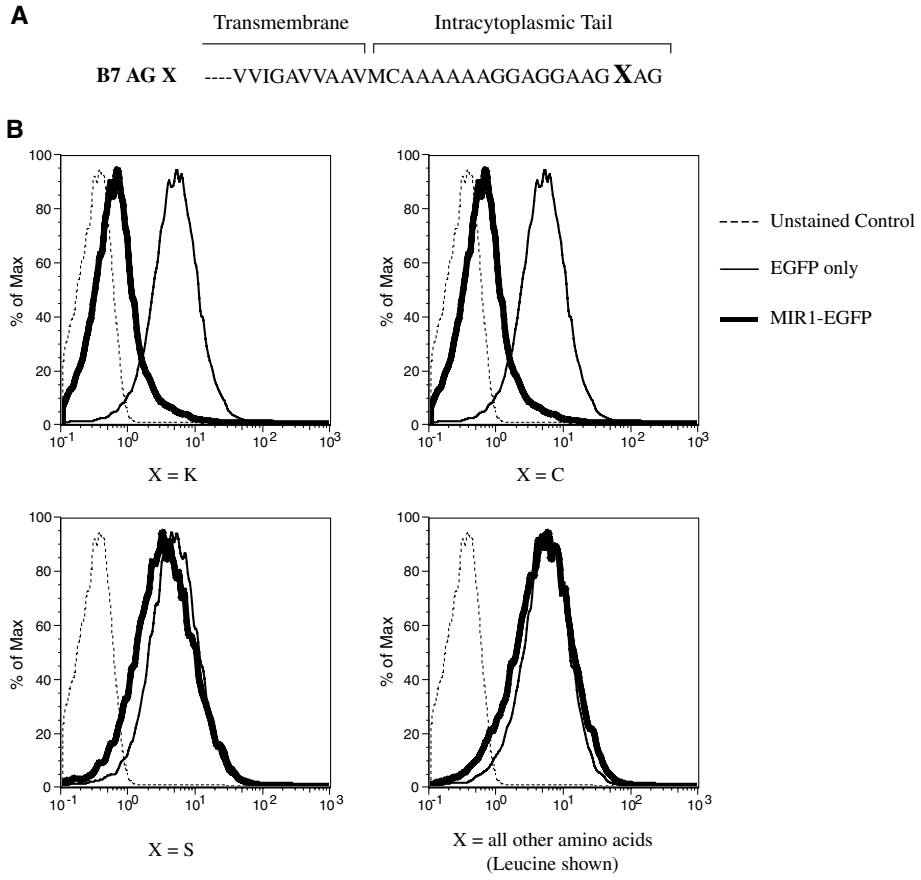

Fig. 3. One lysine or cysteine residue is sufficient to promote down-regulation of HLA.B7 by MIR1. (**A**) Amino acid sequence of the intracytoplasmic tail of HLA.B7 mutants where the tail has been replaced by a random GA stretch. Each of the 20 amino acids was substituted at position X. (**B**) BJAB cells stably expressing the HLA.B7 GA mutants were transfected with MIR1-EGFP and an EGFP control, then analyzed for surface expression of HLA.B7 using flow cytometry.

tination. The physiological relevance, for the virus, of this alternate form of ubiquitination is still unclear. An attractive hypothesis is that the ability of MIR1 to act on lysineless molecules allows it to broaden its potential targets.

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Materials and Methods
SOM Text
Figs. S1 and S2

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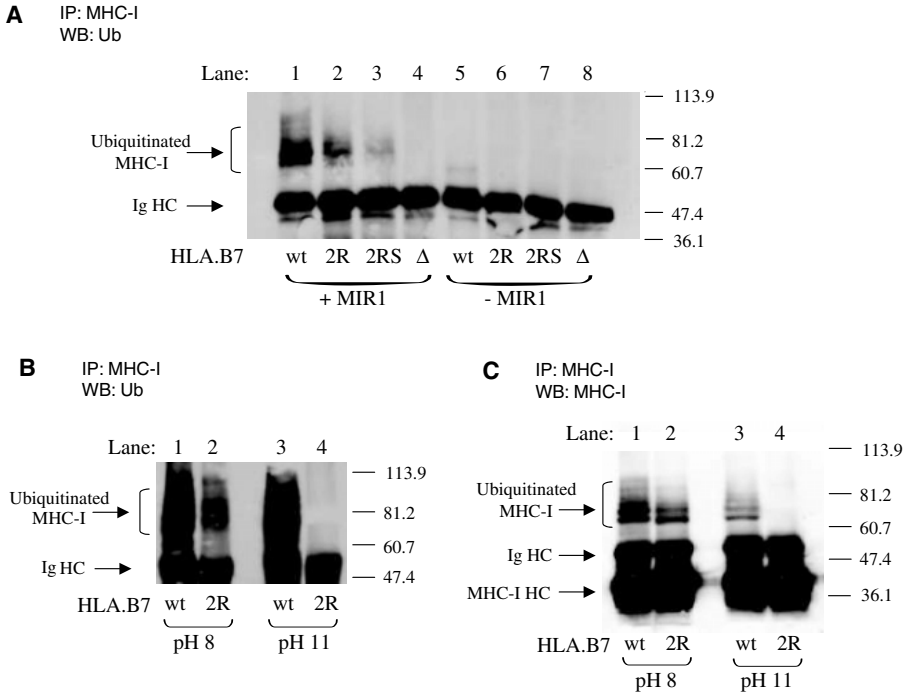
Fig. 4. A novel form of ubiquitination is detectable on HLA.B7 2R. (**A**) Lysates from CHO cells stably expressing wt HLA.B7, HLA.B7 2R, HLA.B7 2RS, and HLA.B7 Δ with or without stable expression of MIR1 were used in an immunoprecipitation reaction. The reaction was carried out using the antibody against human MHC I w6/32 (which recognizes only properly folded human MHC I molecules), and ubiquitinated species were detected by Western blot with an antibody against ubiquitin. (**B**) Lysates from CHO cells stably expressing wt HLA.B7 or HLA.B7 2R with MIR1 were immunoprecipitated with an antibody against MHC I, eluted in the presence of the reducing agent β_2-mercaptoethanol at either pH 8 or pH 11, and analyzed by Western blot using an antibody against ubiquitin. (**C**) Wt HLA.B7 and HLA.B7 2R were immunoprecipitated as above, and the presence of HLA.B7 was determined by staining with the antibody against human MHC I, HC10.



Genome of the Host-Cell Transforming Parasite *Theileria annulata* Compared with *T. parva*

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Theileria annulata and *T. parva* are closely related protozoan parasites that cause lymphoproliferative diseases of cattle. We sequenced the genome of *T. annulata* and compared it with that of *T. parva* to understand the mechanisms underlying transformation and tropism. Despite high conservation of gene sequences and synteny, the analysis reveals unequally expanded gene families and species-specific genes. We also identify divergent families of putative secreted polypeptides that may reduce immune recognition, candidate regulators of host-cell transformation, and a *Theileria*-specific protein domain [frequently associated in *Theileria* (FAINT)] present in a large number of secreted proteins.

Theileria are the only intracellular eukaryotic pathogens capable of reversibly transforming their host cells. *Theileria annulata* (TA) and *T. parva* (TP) are tick-borne hemoparasites (*1*) that give rise to lymphoproliferative diseases (*2*) of cattle known, respectively, as tropical theileriosis and East Coast fever (ECF). The molecular mechanisms are unknown, but previous analyses indicate that both species subvert the same host-cell signal transduction pathways (*3*). Although the parasites have similar life cycles involving intracellular stages in leukocytes and in red blood cells, they are transmitted by different tick species and transform different cell types. In contrast to ECF, cases of tropical theileriosis are accompanied by severe anemia. Available therapeutics are reliable only in the early stages of disease, and existing vaccines rely on the administration of live parasites. There is an urgent need for improved control and therapeutics.

The nuclear genome (*4*) of TA is similar in size (8.35 Mb) to that of TP (8.3 Mb); it spans four chromosomes that range from 1.9 to 2.6 Mb (Table 1 and table S1). We predicted 3792 putative protein-coding genes in TA. In addition, a total of 49 tRNA and 5 ribosomal RNA (rRNA) genes were found, revealing common features in rRNA units between the species (*5*) (table S1). The telomeres and presumptive centromeres of TA and TP are similar in base composition, size, and arrangement.

Like many parasitic protozoa, both *Theileria* spp. have tandem arrays of genus-specific, hypervariable gene families (6) (table S3) that map adjacent to the telomeres (*6*) with an overall arrangement that appears conserved (Fig. 1). Most of these subtelomeric genes encode predicted secreted proteins. Genes previously described as related to the restriction enzyme *Sfi*I fragment (designated family 3, table S3) are found proximal to the telomeres (Fig. 1B), followed by Pro/Gln-rich proteins (family 1, table S3). The boundary between subtelomeric gene families and "housekeeping" genes is defined by adenosine 5′-triphosphate–binding cassette (ABC) transporter genes (family 5, table S3) in the opposite coding orientation. Stage-specific expressed sequence tags (ESTs) indicate that at least three subtelomeric ABC transporters are constitutively transcribed in macroschizont, merozoite, and piroplasm stages in the mammalian host. Members of gene families 3 and 5 also occur internally in the genome. Our findings are consistent with vigorous genetic exchange between subtelomeres, fostering expansion and diversification of antigens, with internal clusters that may act as reservoirs.

The nonsubtelomeric regions of the TA and TP genomes show strong conservation of synteny with only a few inversions of small sequence blocks and no interchromosomal rearrangements (Fig. 1A). Short interruptions to synteny corresponded to the insertion or deletion of genes, and usually involve members of large gene families, as exemplified by the TP repeat (*Tpr*) genes (*4*) and their *Tpr*-related counterparts in TA (*Tar*). These *Tar* genes form the second largest family in both genomes. The majority of *Tpr* genes form a single array on TP chromosome 3 (*5*, *7*), located at a large inversion point. *Tar* genes are dispersed throughout the four chromosomes in TA and cause small interruptions in synteny. The lower sequence divergence between *Tpr* compared with *Tar* genes suggests

Table 1. Comparison of protein coding genes in *T. annulata* and *T. parva*. Unique genes are calculated by filtering the genes without orthologs; members of gene families with counterparts in both genomes are removed, as are any that have a translated query versus translated database (TBLASTX) hit in the other species (*e* value $< 1 \times 10^{-10}$). Genes smaller than 100 amino acids were manually checked.

	T. annulata	*T. parva*
Genome size	8351610	8308027
G+C content	32.54	34.1
Gene number	3792	4035
Genes with orthologs	3265	3265
Genes without orthologs	493	710
Unique genes	34	60

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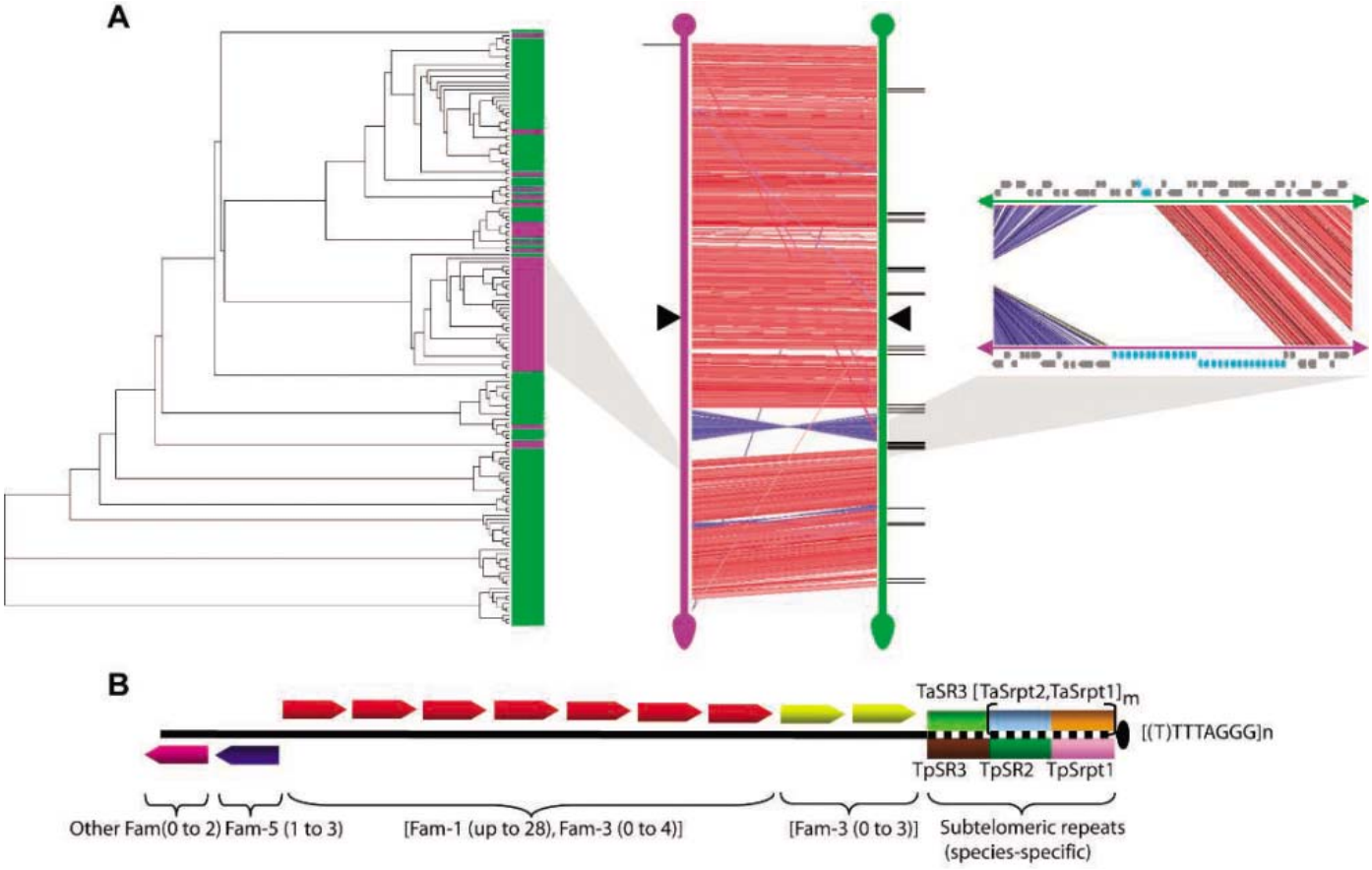


Fig. 1. Large-scale synteny between *T. annulata* and *T. parva* chromosomes. (**A**) Synteny breaks of chromosome 3 of TA (green) and TP (purple) are located at *Tpr* genes. (Middle) Chromosome 3 of TA and chromosome 3 of TP are aligned. Connecting lines show maximal unique matches between the two chromosomes. Red lines, alignments in the same orientation; blue lines, alignments in opposing orientations; black triangles, putative centromeres; black lines, *Tpr* genes occurring outside the *Tpr* locus. The position of the *Tpr* locus of TP is aligned with the gray shaded area. (Left) The phylogenetic tree shows the clustering of the TP genes when compared with the TA genes. Branches ending in green boxes represent TA genes and purple boxes represent TP genes. All genes in the *Tpr* locus occur in the cluster which is aligned with the gray shaded area. (Right) A close-up of the insertion of the *Tpr* locus in TP (purple) with respect to TA (green), with *Tpr* and *Tar* genes (blue) and all other genes (gray). (**B**) Organization of a representative subtelomere (not to scale). The black line represents the coding part of the subtelomere, with the arrangement of gene families (arrowheads) shared between TA and TP. The arrowheads indicate the transcriptional orientation; the observed range in numbers of genes is in parentheses. The dotted black line represents the species-specific noncoding regions (upper, TA; lower, TP). Srpts, subtelomeric repeats; SR, subtelomeric regions (*4*).

that they expanded after speciation. The single array in TP may allow gene conversion to prevent divergence.

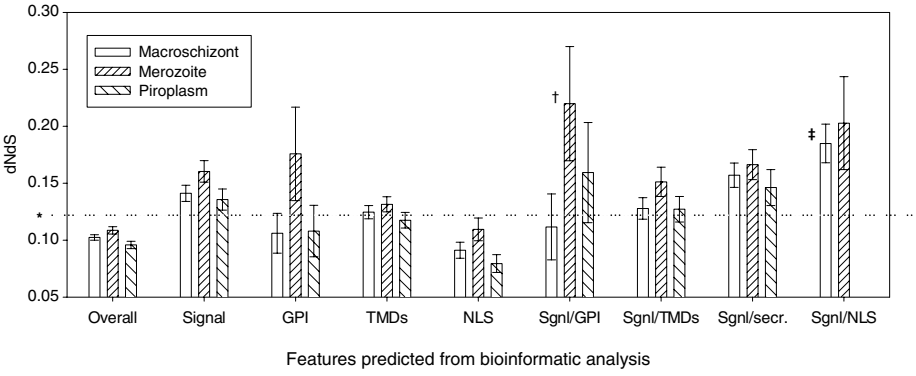
Noncoding regions of subtelomeres are complex. In TA, from the terminus inward, a succession of paired guanine-cytosine (GC)–rich subtelomeric repeats (TaSrpt1 and TaSrpt2) are followed by a single-copy sequence at all chromosome ends (TaSR3; Fig. 1B and fig. S3). No such repeats are found in TP subtelomeres; a terminal sequence (TpSrpt1, ~140 base pairs) is shared by all chromosomal ends, followed by a thymine-rich region (TpSR2), then by a region shared by many but not all TP subtelomeres (TpSR3).

We predicted 3265 orthologous genes between the genomes. Most genes without orthologs are members of gene families; only a small proportion (34 in TA, 60 in TP; table S4) are single-copy genes to which functions could not be ascribed, but EST data detected that four of these are expressed in TA. No major species differences were found in the numbers of predicted transcription-associated proteins, peptidases (*4*), or core metabolic enzymes (*5*).

We evaluated evolutionary pressure acting on genes using the ratio of nonsynonymous to synonymous substitutions (dN/dS) between orthologs (table S7). This method can potentially identify immunogenic genes and thus putative vaccine candidates (*8*). Where possible, we matched dN/dS with stage-specific expression patterns from the EST data in TA. Constitutively expressed genes displayed the lowest dN/dS values (Fig. 2). Similar to *Plasmodium* (*9*), genes encoding merozoite surface proteins yielded the highest dN/dS ratios (Fig. 2); these proteins are candidates for immune selection (*10*). For predicted macroschizont polypeptides with signal peptides, dN/dS values were also high, although lower than those for merozoites. Surprisingly, genes encoding macroschizont glycosylphosphatidylinositol (GPI)–anchored membrane proteins have dN/dS values similar to housekeeping genes. In contrast, high dN/dS ratios were found for macroschizont proteins without predicted membrane retention motifs that are potentially secreted into the leukocyte cytosol. The high dN/dS values associated with host-exported *Theileria* proteins might reflect regulatory functions that have diversified after speciation of TA and TP. Alternatively, they might reflect exposure to the immune system, after rapid degradation to generate peptides presented by major histocompatibility complex antigens on the infected cell surface. Consistent with this, PEST (a signal for rapid proteolytic degradation) regions (*11*) were identified in many of these polypeptides (table S8).

Almost all members of the major *Theileria*-specific subtelomeric protein family members incorporate varying numbers (1 to 54) of a single, highly polymorphic domain with an average length of 70 residues, a designation frequently associated in *Theileria* (FAINT), formerly known as DUF529 (*12*). Over 900 copies were found in 166 TA proteins and in equivalent numbers of TP proteins (fig. S5).

A



B

Protein-coding regions	
Av % protein ID	83.7
Av % nucleotide ID	82.4
Av dN	0.09
Av dS	0.82
Av dN/dS	0.097
Non-protein coding regions	
% nucleotide ID	74

Fig. 2. (**A**) dN/dS ratios computed between pairs of orthologous genes in TA and TP. Mean dN/dS values of expressed proteins as a function of life-cycle stage in TA and predicted protein motifs and signals. Error bars show means ± SE. EST data were from cDNAs from three life-cycle stages in TA (macroschizont, merozoite, and piroplasm). Grouping of proteins was based on presence of certain domains (*4*), indicated as follows: Signal, presence of a signal peptide; GPI, GPI anchor; TMD, transmembrane domain; NLS, nuclear localization sequence; secr., secreted. We assume where GPIs occurred in the absence of signal peptides, it was because of the limitations of gene boundaries and in the prediction software. Dotted line marked by asterisk, 0.1220, average dN/dS across all genes with orthologs; †, merozoite/signal/GPI proteins versus other merozoite proteins (P = 0.016; 95% CI: 0.0214 to 0.2080), Mann-Whitney test; ‡, macroschizont/signal/NLS proteins versus other macroschizont proteins (P = 0.001; 95% CI: 0.04831 to 0.13320), Mann-Whitney test. (**B**) Summary of the analysis. The average (Av) dN/dS ratios and identities (ID) of coding and noncoding regions are shown for all orthologous genes between TA and TP.

The majority of the FAINT domain–containing proteins have no other recognizable domains except a putative signal peptide, consistent with export to the host. However, in members of the TashAT gene cluster, one or more FAINT domains appear with AT-hook and PEST motifs on the same protein (*13*, *14*) (fig. S5 and table S8). We found only one other FAINT domain containing protein in the UniProt protein database (*15*), occurring in a nontransforming *Theileria* (synonym of *Babesia equi*), which also invades leukocytes and develops to a macroschizont stage (*16*). We also described proteins containing previously unrecognized short amino acid repeat domains in both genomes (*4*). The species-specific nature of the domains suggests that they have expanded recently (*4*) (fig. S1).

The parasite genes involved in host-cell transformation must be expressed by the macroschizont stage, and their products must be released into the host cell cytoplasm or expressed on the parasite surface. This would generally require a signal peptide or a specific host-targeting signal sequence. Candidates meeting these criteria include the previously described TashAT and SuAT protein families in TA (*13*, *14*) and related TP host nuclear proteins (TpHNs) in TP. In addition to localizing to the host nucleus, members of the TashAT family bear cyclin-dependent kinase phosphorylation motifs, cyclin docking sites, and AT-hook DNA binding domains (table S8). A cluster of 17 SuAT1- and TashAT-like genes was identified in the TA genome and an orthologous gene family of 20 members in a syntenic region of the TP genome. However, TpHNs lack consensus AT-hook motif, a divergence that could be interpreted as a result of species adaptation to their preferred host-cell type.

We screened both predicted proteomes with a database of proteins linked to cell transformation and tumor progression (*17*) and matched the hits with the presence of a signal peptide and the macroschizont EST data set (*4*). No obvious proto-oncogenes, kinases, or phosphatases were identified. However, this screen did identify members of the HSP90 subfamily, DEAD-box RNA helicases, peptidases, immunophilins, members of the thioredoxin/glutaredoxin family, and leucine-zipper proteins (table S9).

Proteins that function in lipid metabolism were also identified as transformation candidates. First, we found proteins related to phospholipase A2, whose activity is elevated in tumor cells (*18*), in both predicted proteomes and, unlike in other apicomplexan parasites, they carry a signal peptide. Second, choline kinase genes (ChoKs) are present at high copy number compared with other apicomplexans. ChoK activity is deregulated in transformed cell lines and its inhibition results in a reversible blockage of cell proliferation (*19*). Finally, the cell cycle effectors uridine phosphorylases and leucine carboxyl methyltransferases (*20*), whose activity is raised in tumor cells (*21*), are tandemly duplicated in TA and TP. However, no signal sequence is predicted for the latter three enzymes, so it remains to be determined whether their expansion reflects the ability of the macroschizont to maintain host-cell transformation.

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Supporting Online Material
www.sciencemag.org/cgi/content/full/309/5731/131/DC1
Materials and Methods
Figs. S1 to S5
Tables S1 to S9
References

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Genome Sequence of *Theileria parva*, a Bovine Pathogen That Transforms Lymphocytes

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We report the genome sequence of *Theileria parva*, an apicomplexan pathogen causing economic losses to smallholder farmers in Africa. The parasite chromosomes exhibit limited conservation of gene synteny with *Plasmodium falciparum*, and its plastid-like genome represents the first example where all apicoplast genes are encoded on one DNA strand. We tentatively identify proteins that facilitate parasite segregation during host cell cytokinesis and contribute to persistent infection of transformed host cells. Several biosynthetic pathways are incomplete or absent, suggesting substantial metabolic dependence on the host cell. One protein family that may generate parasite antigenic diversity is not telomere-associated.

Theileria parva is a tick-borne parasite that causes a fatal disease in cattle known as East Coast fever (ECF). This disease, which kills over 1 million cattle each year in sub-Saharan Africa, results in economic losses exceeding $200 million annually (*1*). *Theileria* organisms belong to the phylum Apicomplexa, which is predicted to have originated about 930 million years ago (*2*). Unlike other apicomplexans, penetration of host cells by *T. parva* is not orientation-specific. Rhoptries and microspheres discharge after invasion, coincident with dissolution of the surrounding host cell membrane, leaving the parasite free in the host cell cytoplasm. Morbidity and mortality due to ECF are attributed to the ability of the schizont stage to malignantly transform its host cell, the bovine lymphocyte. Parasitosis increases exponentially because the schizont divides in synchrony with the host cell and infected cells infiltrate all tissues; cattle die of this lymphoproliferative disease 3 to 4 weeks after infection. Little pathology is due to the tick infective piroplasm, the red blood cell stage (*1*).

We sequenced the genome of *T. parva* in order to facilitate research on parasite biology, assist the identification of schizont antigens for vaccine development (*3*), and extend comparative apicomplexan genomics, in particular with *Plasmodium falciparum*, which causes malaria. Comparison with *T. annulata,* which causes tropical bovine theileriosis and mainly transforms macrophages, is described in an accompanying report (*4*). (This whole-genome shotgun project has been deposited at DNA Data Bank of Japan/European Molecular Biology Laboratory/GenBank under the project accession AAGK00000000.)

The haploid *T. parva* nuclear genome is 8.3×10^6 base pairs (Mbp) in length and consists of four chromosomes (Table 1). We provide a complete sequence, except for a 1- to 2-kbp gap in chromosome 4 and a gap in chromosome 3 (Tpr locus) that contains a 41-kbp and a 13-kbp set of overlapping sequences (contig) (*5*). The parasite apicoplast and mitochondrial (*6*) genomes have also been sequenced. Like *P. falciparum*, *T. parva* chromosomes contain one extremely A+T-rich region (>97%) about 3 kbp in length that may be the centromere. The regions between the $CCCTA_{3\text{-}4}$ telomeric repeats and the first protein-encoding gene are short, 2.9 kbp on average, and do not contain other repeats. Thus, the structure of the subtelomeric regions in *T. parva* is much less complex than that in *P. falciparum*, where arrays of repeats extend up to 30 kbp (*7*).

The *T. parva* nuclear genome contains about 4035 protein-encoding genes, 20% fewer than *P. falciparum*, but exhibits higher gene density, a greater proportion of genes with introns, and shorter intergenic regions. There are two identical, unlinked 5.8S-18S-28S rRNA units, suggesting that unlike *P. falciparum T. parva* does not possess functionally distinct ribosomes (*8*). Putative functions were assigned to 38% of the predicted proteins (Table 1).

The complexity of the *T. parva* life cycle is not matched by a large number of recognizable cell cycle regulators. Thus, the parasite is more akin to yeasts than higher eukaryotes, lacking discernable components of both the p53-MDM2-p14ARF-p21 and the Ink4-retinoblastoma-E2F pathways (*9*). There are four predicted cyclins and five cyclin-dependent kinases (cdks), most of which have close homologs in *P. falciparum*. However, *T. parva* lacks one cyclin and two cdks found in *P. falciparum*. These parasite cyclins are poorly conserved (~25% identity), making cross-species comparisons difficult. The reduced recognizable *T. parva* cell cycle machinery suggests that a number of novel regulatory features remain to be discovered.

A unique aspect of *T. parva* biology is that infection of T and B lymphocytes results in a reversible transformed phenotype with uncontrolled proliferation of host cells that remain persistently infected. Parasite proteins that may modulate host cell phenotype are described in an accompanying report (*4*). Host cell microtubules that decorate the surface of schizonts are captured by the host cell spindle during mitosis, favoring infection of both daughter cells (*1*). *T. parva* encodes putative secreted forms of EMAP115- and Tau-like proteins, which are absent from *P. falciparum*; in higher eukaryotes, these proteins interact with microtubules (*10*). In addition, *T. parva* may modulate host cell mitosis by influencing disassembly of the host cell spindle via a secreted cdc48-like AAA–adenosine triphosphatase

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(ATPase associated with diverse cellular activities) (*11*). A likely *P. falciparum* homolog of this protein contains an N-terminal signal anchor sequence, whereas the *T. parva* protein contains a signal peptide and lacks a recognizable endoplasmic reticulum retention signal.

We used the Tribe-MCL algorithm (*5*) to identify 384 protein families containing 1063 proteins in the *T. parva* proteome (table S1). The largest family, containing 85 proteins, exists primarily in tandem arrays in the subtelomeric regions of all chromosomes. Many members of the family have a similar architecture, consisting of a secretion signal at the N terminus and a low-complexity glutamine- and proline-rich central domain that may be difficult for vertebrate immune systems to recognize (*12*). These genes are polymorphic between parasite isolates, and specific genes are absent from certain isolates (*13*). Each telomere has a conserved ~140-bp sequence immediately adjacent to the telomeric repeat (*14*), and several subtelomeric regions exhibit 70 to 100% sequence similarity (fig. S1). As in other eukaryotic pathogens, these features may facilitate interchromosomal recombination and the generation of antigenic diversity.

Proteins in the most rapidly evolving *T. parva* protein family, the Tpr (*T. parva* repeat) family, contain complex domain structures reminiscent of a system that has evolved to generate diversity (*15*). Unlike the majority of hypervariable gene families in parasitic protozoa (*16*), Tpr sequences are not telomere-associated. This family comprises a tandem array of highly conserved open reading frames (ORFs) on chromosome 3, located ~570 kbp from a telomere. The locus, estimated to span 100 kbp, contains at least 28 ORFs, of which 18, ranging in length from 192 to 674 amino acids, lack methionine codons in the first 50 amino acids (fig. S2). Eleven additional dispersed copies of Tpr, also of varying length, contain a 268–amino acid membrane-associated helical domain typical of the Tpr family. Massively parallel signature sequencing (*17*) and expressed sequence tags suggest that some genes in the locus are only transcribed in the piroplasm stage, whereas at least two of the dispersed genes are transcribed in the schizont stage. In common with the *var* genes of *P. falciparum* (*18*), domains within the Tpr genes are isolate-specific (*19*), and the 3′ end of Tpr has been used for genotyping of *T. parva* isolates. Tpr proteins have not yet been detected in piroplasms, and the function of these proteins remains unknown.

The genome sequence provides a global view of the metabolic potential of *T. parva* and allows a comparative analysis with *P. falciparum* metabolism. We predict a reduced functional role for the *T. parva* apicoplast and a greater dependence on the host for many substrates (fig. S3). *T. parva* lacks many enzymes in the shikimic acid, porphyrin, polyamine, and type II fatty acid biosynthetic pathways, but it retains the ability to produce isoprenoids via a methyl erythritol phosphate pathway in the apicoplast. *T. parva* cannot salvage purines, its ability to interconvert amino acids is very limited, and it lacks enzymes that permit the alternative nonoxidative production of pentoses and tetroses via the pentose phosphate pathway. Analysis of predicted transporters revealed fewer transporters of organic nutrients and inorganic cations than are present in *P. falciparum*. However, *T. parva* has more adenosine 5′-triphosphate-binding cassette (ABC) transporters of unknown substrate specificity. Another difference is that *T. parva* encodes an amino acid–cation symporter that is not present in *P. falciparum* (*7*) or *C. parvum* (*20*). In contrast to *P. falciparum*, *T. parva* encodes trehalose-6-phosphate synthase and trehalose phosphatase. Trehalose is a disaccharide that plays a role in desiccation and stress tolerance. It may protect the parasite during its long developmental cycle in the tick.

T. parva genes encode all of the enzymes necessary for glycolysis, glycerol catabolism, and the tricarboxylic acid (TCA) cycle. Unlike *P. falciparum*, *T. parva* does not encode malate dehydrogenase, but this could be functionally replaced by malate-quinone oxidoreductase, an activity also predicted to be present in *P. falciparum*. The origin of mitochondrial acetyl-coenzyme A (CoA) in both parasites presents a problem, because *P. falciparum* encodes a single pyruvate dehydrogenase that is targeted to the apicoplast (*21*) and *T. parva* does not encode all the subunits of this enzyme. Both parasites are predicted to contain cytoplasmic acetyl-CoA synthetase and a plasma membrane acetyl-CoA–CoA antiporter, but how mitochondrial oxidation of carbon chains is fueled in these two pathogens remains enigmatic because glycolysis and the tricarboxylic acid cycle do not appear to be linked by a classical route (*22*). Thus, it is not clear whether the complete TCA cycle is functional. Nitrogen metabolism differs from *P. falciparum* because *T. parva* lacks glutamate-ammonia ligase and only contains a nicotinamide adenine dinucleotide (NAD^+)–dependent glutamate dehydrogenase, which is usually associated with glutamate catabolism. This suggests that imported glutamate could play a role in supplementing intermediates in the TCA cycle.

The ionophores valinomycin and gramicidin D kill *T. parva*, suggesting that a mitochondrial electrochemical gradient is essential for parasite survival (*23*), but it is not known whether this is coupled to ATP synthesis. All subunits of the F1 catalytic domain of ATP synthase and subunit c of the F0 domain are present, but genes coding for subunits a and b of F0 were not found. The *T. parva* respiratory complexes are similar to those described in *P. falciparum.* Buparvaquone, a hydroxynapthaquinone drug used in the chemotherapy of ECF, probably inhibits electron transport through complex III (*23*).

The apicoplast is found in most apicomplexans and plays an essential role in parasite metabolism (*24*). An A+T-rich, ~35-kbp apicoplast genome encoding 30 proteins, rRNAs, and tRNAs is present in *Plasmodium*, *Toxoplasma*, and *Eimeria*, but not in *Cryptosporidium* (*20*); the latter lacks an apicoplast. The 39.5-kbp *T. parva* apicoplast genome differs from that of *P. falciparum* in that all of its genes are transcribed in the same direction. In addition, it has one rather than two copies of the rRNA genes, *clp*C is duplicated, the *rpoC2* gene encoding the β″ subunit of RNA polymerase is split into two parts, and it lacks the *sufB* gene (Fig. 1). Twenty-six of the 44 *T. parva* apicoplast genome protein-coding genes share sequence

Table 1. Comparison of *T. parva* nuclear genome coding characteristics with other sequenced apicomplexans. Gene length excludes introns; gene density calculated as genome size/number of protein-encoding genes. Source of data for *P. falciparum* was (*7*), and, for *C. parvum*, (*20*).

Features	Apicomplexan organism		
	T. parva	*P. falciparum*	*C. parvum*
Size (bp)	8,308,027	22,853,764	9,100,000
Number of chromosomes	4	14	8
Total G+C content (%)	34.1	19.4	30
Number of protein encoding genes	4035	5268	3807
Number of hypothetical proteins	2498	3208	925
Mean gene length (bp)	1407	2283	1795
Gene density (gene frequency in bp)	2057	4338	2382
Percent coding	68.4	52.6	75.3
Genes with introns (%)	73.6	53.9	5
Exons per gene (median)	4	2	1
Mean intergenic length (bp)	405	1694	566
G+C content intergenic regions (%)	26.1	13.6	23.9
Number of tRNA genes	47	43	45
Number of 5S rRNA genes	3	3	6
Number of rRNA units	2	7	5

similarity (27 to 61%) with proteins encoded by the *P. falciparum* apicoplast genome.

Most apicoplast proteins are encoded by nuclear genes and imported into the organelle by means of a bipartite targeting presequence (*24*). Comparison of the 345 *T. parva* (*5*) and 551 *P. falciparum* (*7*) predicted apicoplast-targeted (AT) proteins revealed similarities and differences in apicoplast function. The apicoplasts of *Plasmodium* and *Toxoplasma* participate in heme biosynthesis and are the sites of type II fatty acid and isoprenoid biosynthesis. Apicoplast-derived fatty acids in these parasites might contribute to the establishment and modification of the parasitophorous vacuole membrane (*25*). It may be notable that both *T. parva* and *T. annulata*, which have only retained isoprenoid biosynthesis, do not exist within a parasitophorous vacuole. About 100 AT proteins were found in both species, but 40% of these were hypothetical proteins, indicating that many core apicoplast functions have yet to be defined.

Fe-S clusters are required in mitochondria and plastids for the maturation of apoproteins. Fe-S cluster formation in the *T. parva* mitochondrion appears to be similar to that in yeast and *Plasmodium* (*26*) (table S3). However, of the *sufABCDES* genes involved in the assembly of Fe-S clusters in *Arabidopsis thaliana* (*27*) and *P. falciparum* plastids (*26*), only *sufS* was identified in *T. parva*. SufS is a cysteine desulfurase that requires SufE for catalytic activity. The parasite *T. para* genome encodes a plastid-targeted tRNA thiolation enzyme (MnmA) that has an additional domain similar both in sequence and predicted structure to the sulfur-binding domain of SufE. Thus, a previously unknown complex of SufS/MnmA may catalyze thiolation of tRNA in the *T. parva* apicoplast. The *T. parva* nuclear genome also encodes an AT protein with homology to NFU1, a scaffold protein for Fe-S cluster assembly in *A. thaliana* plastids (*28*), suggesting that assembly of Fe-S clusters occurs in the *T. parva* apicoplast despite the absence of most Suf proteins.

T. parva and *T. annulata* exhibit near-complete synteny across all chromosomes (*4*). To examine the extent of conservation of gene synteny between the evolutionarily distant *P. falciparum* and *T. parva*, we applied an iterative syntenic block algorithm and Jaccard-filtered COGs to whole-genome data from *P. falciparum* clone 3D7 (*7*), *P. y. yoelii* (*29*), *C. parvum* (*20*), and *T. parva*. Extensive synteny was found between *P. falciparum* and *P. y. yoelii* but not between *P. falciparum* and *C. parvum* or between *T. parva* and *C. parvum*. A total of 435 microsyntenic regions containing 1279 orthologs were observed between *P. falciparum* and *T. parva*, consisting of groups of 2 to 11 orthologs conserved in position between the two genomes (Fig. 2). This may be an underestimate of the degree of microsynteny as it is possible that, due to its long-term in vitro culture, clone 3D7 may represent an atypical genome. Syntenic clusters were distributed uniformly along each chromosome except for the subtelomeric regions, which contain species-specific gene families.

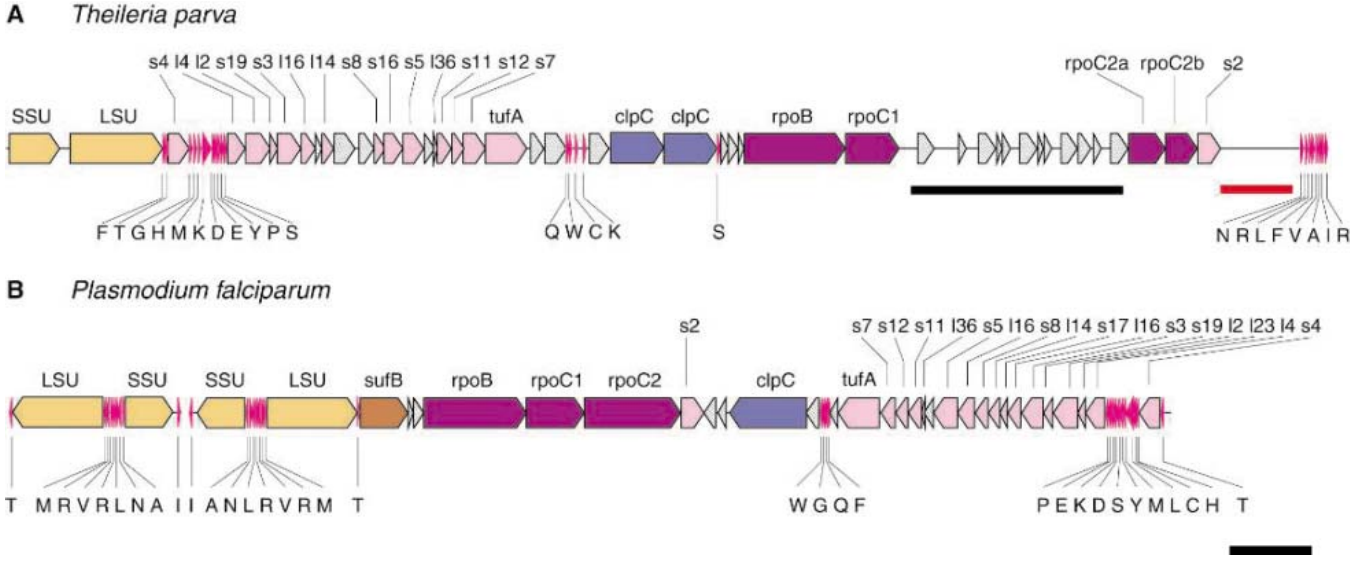

Fig. 1. Comparison of the apicoplast genomes of *T. parva* (**A**) and *P. falciparum* (**B**). A circular contig of the *T. parva* apicoplast genome was obtained after assembly of shotgun sequences, but the in vivo conformation has not been determined. The *P. falciparum* apicoplast genome is circular in vivo (*30*). The genomes are displayed in linear format beginning with the small subunit rRNA genes. Abbreviations and color coding: light orange, small (SSU) and large (LSU) subunit rRNAs; magenta, tRNAs [single-letter amino acid code (*31*)]; pink, ribosomal proteins (s and l for small and large subunit ribosomal proteins, respectively) and elongation factor Tu (tufA); blue, protein import; stippled gray, hypothetical proteins; purple, transcription; brown, SufB subunit of the SufABCDE Fe-S cluster assembly complex. The black and red bars indicate a region containing repeats and short ORFs and another region containing repeats and potential selenocysteine tRNAs, respectively (*5*). Scale bar equals 1 kbp.

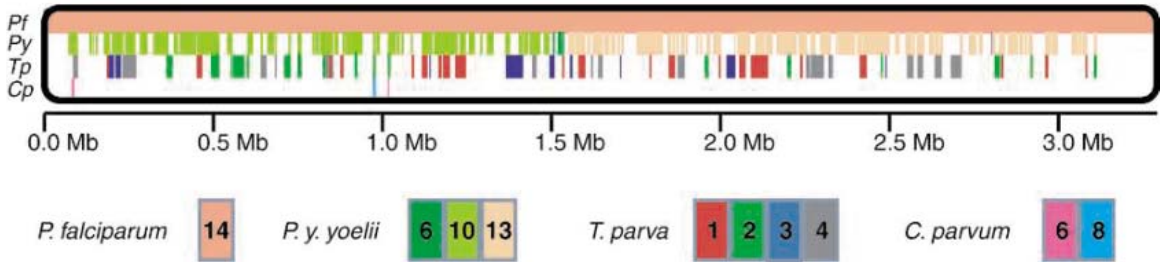

Fig. 2. Regions of microsynteny between *T. parva* and *P. falciparum*. Schematic of a representative *P. falciparum* chromosome showing synteny with three other apicomplexan species. Top row, *P. falciparum* chromosome 14 proteins. Second row, *P. y. yoelii* orthologs from *P. y. yoelii* chromosomes 6, 10, and 13. Third row, *T. parva* orthologs from *T. parva* chromosomes 1, 2, 3, and 4. Fourth row, *C. parvum* orthologs from *C. parvum* chromosomes 6 and 8.

The genome sequence of *T. parva* shows remarkable differences from the other apicomplexan genomes sequenced to date. It provides significant improvements in our understanding of the metabolic capabilities of *T. parva* and a foundation for studying parasite-induced host cell transformation and constitutes a critical knowledge base for a pathogen of significance to agriculture in Africa. Mining of sequence data has already proved useful in the search for candidate vaccine antigens (*3*).

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Long-Term Monitoring of Bacteria Undergoing Programmed Population Control in a Microchemostat

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Using an active approach to preventing biofilm formation, we implemented a microfluidic bioreactor that enables long-term culture and monitoring of extremely small populations of bacteria with single-cell resolution. We used this device to observe the dynamics of *Escherichia coli* carrying a synthetic "population control" circuit that regulates cell density through a feedback mechanism based on quorum sensing. The microfluidic bioreactor enabled long-term monitoring of unnatural behavior programmed by the synthetic circuit, which included sustained oscillations in cell density and associated morphological changes, over hundreds of hours.

By continually substituting a fraction of a bacterial culture with sterile nutrients, the chemostat (*1*, *2*) presents a near-constant environment that is ideal for controlled studies of microbes and microbial communities (*3*–*6*). The considerable challenges of maintaining and operating continuous bioreactors, including the requirement for large quantities of growth media and reagents, have pushed the move toward miniaturization and chip-based control (*7*–*10*), although efforts have been limited to batch-format operation. Microbial biofilms, which exist in virtually all nutrient-sufficient ecosystems (*11*), interfere with continuous bioreactor operation (*12*). Phenotypically distinct from their planktonic counterparts (*11*), biofilm cells shed their progeny into the bulk culture and create mixed cultures. At high dilution rates, the biofilm, which is not subject to wash-out, supplies most of the bulk-culture cells (*13*). The increase in surface area-to-volume ratio as the working volume is decreased aggravates these wall-growth effects (*13*).

We created a chip-based bioreactor that uses microfluidic plumbing networks to actively prevent biofilm formation. This device allows semicontinuous, planktonic growth in six independent 16-nanoliter reactors with no observable wall growth (Fig. 1A). The cultures can be monitored in situ by optical microscopy to provide automated, real-time, noninvasive measurement of cell density and morphology with single-cell resolution.

Each reactor, or "microchemostat," consists of a growth chamber, which is a fluidic loop 10 μm high, 140 μm wide, and 11.5 mm in circumference, with an integrated peristaltic pump and a series of micromechanical valves to add medium, remove waste, and recover cells (Fig. 1B). The growth loop is itself composed of 16 individually addressable segments. The microchemostat is operated in one of two alternating states: (i) continuous circulation, and (ii) cleaning and dilution. During continuous circulation, the peristaltic pump moves the microculture around the growth loop at a linear velocity of $\sim$250 μm s^{-1} (Fig. 1C). During cleaning and dilution, the mixing is halted and a segment is isolated from the rest of the reactor with micromechanical valves. A lysis buffer is flushed through the isolated segment for 50 s to expel the cells it contains, including any wall-adhering cells (Fig. 1D). Next, the segment is flushed with sterile growth medium to completely rinse out the lysis buffer. This segment, filled with sterile medium, is then reunited with the rest of the growth chamber, at which point continuous circulation resumes. This process is repeated sequentially on different growth chamber segments, thus eliminating biofilm formation and enabling pseudocontinuous operation. In comparison, passive treatment of the microfluidic surfaces with nonadhesive surface coatings [such as poly (ethylene glycol), ethylenediaminetetraacetic acid, polyoxyethylene sorbitan monolaurate, and bovine serum albumin] proved ineffective in preventing biofilm forma-

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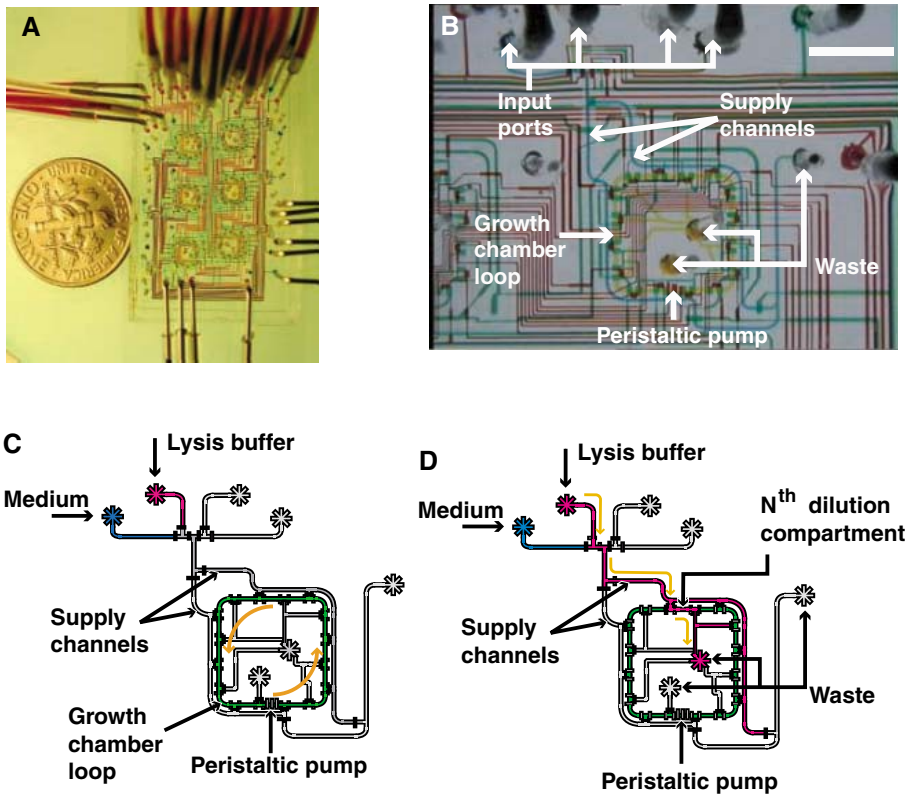

Fig. 1. (**A**) Optical micrograph showing six microchemostats that operate in parallel on a single chip. Various inputs have been loaded with food dyes to visualize channels and sub-elements of the microchemostats. The coin is 18 mm in diameter. (**B**) Optical micrograph showing a single microchemostat and its main components. Scale bar, 2 mm. (**C**) Schematic diagram of a microchemostat in continuous circulation mode. Elements such as the growth loop with individually addressable connected segments, the peristaltic pump, supply channels, and input/output ports are labeled. (**D**) Isolation of a segment from the rest of the growth chamber during cleaning and dilution mode. A lysis buffer (indicated in red) is introduced into the chip through the lysis buffer port. Integrated microvalves direct the buffer through the segment, flushing out cells, including those adhering to chamber walls. The segment is then rinsed with fresh sterile medium and reunited with the rest of the growth chamber.

tion. Without active removal, biofilms invaded the fluidic channels within ~48 hours (*14*).

We performed more than 40 growth experiments with *Escherichia coli* MG1655 cells in five different chips, using a variety of growth media ([MOPS EZ Rich (Teknova, Inc.)] and LB broth with various concentrations of glucose and bacto-tryptone) at 21°C and 32°C. Upon inoculation, a typical culture began with a short lag period, followed by an exponential growth phase that gave way to a steady-state regime (Fig. 2A). Steady-state growth was achieved over a range of dilution rates (0.072 to 0.37 $hour^{-1}$); wash-out was observed at high dilution rates. The steady-state cell concentrations scaled with dilution rate and nutrient richness, decreasing with increasing dilution rates or decreasing bacto-tryptone concentration (Fig. 2B).

To demonstrate the ability of the microchemostat to facilitate analysis of complex growth dynamics, we used it to monitor the dynamics of cell populations containing a synthetic "population control" circuit (*15*), which autonomously regulates the cell density through a negative feedback system based on quorum sensing (*16*). With the circuit ON, the cell density is broadcast and detected through the synthesis and sensing of a signaling molecule (acyl-homoserine lactone, or AHL), which in turn modulates the expression of a killer gene (*lacZα-ccdB*). The killer gene regulates cell density by controlling the cell death rate. The circuit, under control of a synthetic promoter, is inducible with isopropyl-β-D-thiogalactopyranoside (IPTG) (fig. S1). The population control circuit's use of cell-cell communication enables the programming of a bacterial population such that the circuit is insulated from noise in gene expression as

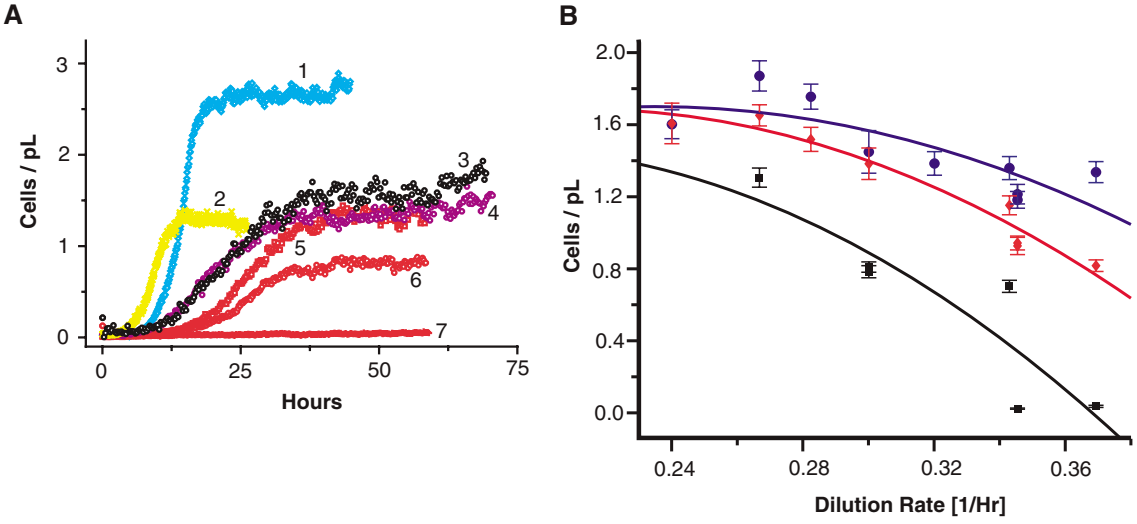

Fig. 2. (**A**) Bacterial growth as a function of time in various media and dilution rates *D*: (1) MOPS EZ Rich, in 1.1 M glucose, $D = 0.34\ hour^{-1}$, at 32°C; (2) MOPS EZ Rich, in 0.11 M glucose, $D = 0.30\ hour^{-1}$, at 32°C; (3) LB, in 0.5 g/L of bacto-tryptone, $D = 0.24\ hour^{-1}$, at 21°C; (4) LB, in 0.5 g/L of bacto-tryptone, $D = 0.30\ hour^{-1}$, at 21°C; (5) LB, in 3 g/L of bacto-tryptone, $D = 0.37\ hour^{-1}$, at 21°C; (6) LB, in 0.5 g/L of bacto-tryptone, $D = 0.37\ hour^{-1}$, at 21°C; and (7) LB, in 0.1 g/L of bacto-tryptone, $D = 0.37\ hour^{-1}$, at 21°C. The red curves (5, 6, and 7) represent different concentrations of bacto-tryptone in LB at a fixed dilution rate, and the open circles (3, 4, and 6) represent constant influent nutrient composition at various dilution rates. Cultures 3 to 7 were cultivated on a single chip, whereas cultures 1 and 2 were each cultivated on separate chips. (**B**) Graph showing steady-state populations for various choices of dilution rate and nutrient concentrations. The error bars represent the variation in the measured steady-state cell density. The steady-state concentration decreases as the dilution rate increases and increases in proportion to the influent nutrient richness. Black squares, 0.1 g/L of bacto-tryptone; red diamonds, 0.5 g/L of bacto-tryptone; blue circles, 3 g/L of bacto-tryptone.

well as intercellular phenotypical variability. This circuit has been characterized in detail for macroscopic cultures (*15*), which makes it particularly appropriate for evaluating performance of the microchemostat in a well-controlled manner.

A microchemostat chip was used to perform six simultaneous experiments with *E. coli* MC4100Z1 cells and a dilution rate of 0.16 hour^{-1} (Fig. 3A). Cultures in reactors 1 to 3 with circuit-bearing cells were induced with IPTG (circuit ON), whereas those in 5 and 6 were not induced (circuit OFF). Reactor 4 contained a circuit-free population with IPTG. Circuit-free and circuit-OFF cultures (4, 5, and 6) grew exponentially to a steady-state density of ~3.5 cells/pL. In contrast, circuit-ON populations (1, 2, and 3) exhibited oscillatory dynamics before reaching a lower steady-state population density after ~125 hours. Variations in cell density due to the discretized nature of the microchemostat dilution scheme were negligible compared to the overall population fluctuations (Fig. 3B).

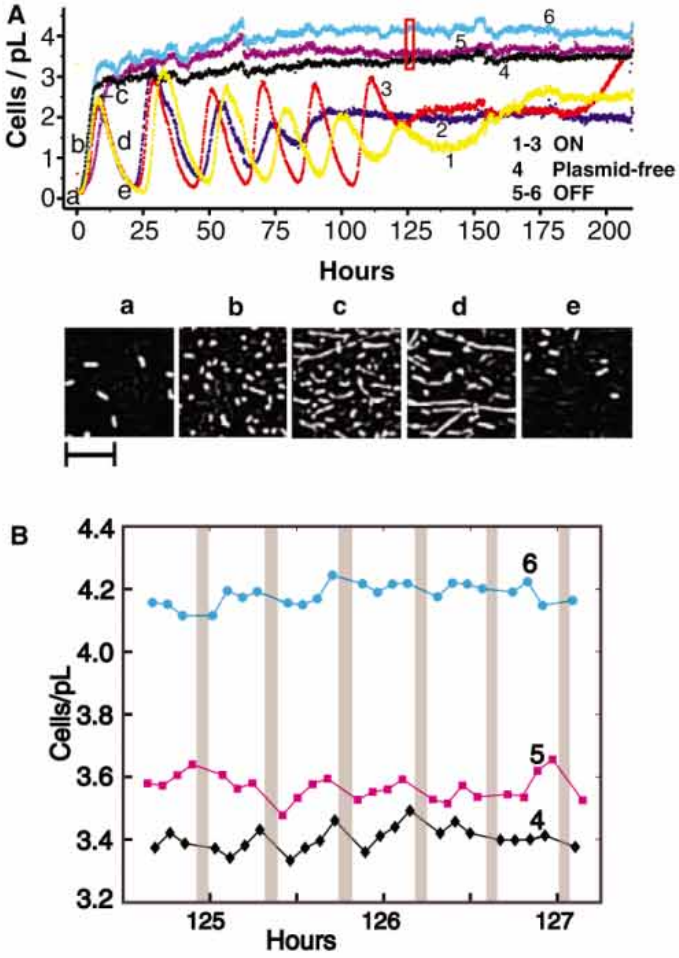

Fig. 3. (**A**) Growth of MC4100Z1 cells with the population-control circuit ON (reactors 1 to 3), OFF (reactors 5 to 6), or absent (reactor 4) on a single chip. Bottom panels (a to e) show micrographs of the culture in reactor 3 at the corresponding points during the first oscillation (scale bar, 25 μm). Cells were grown at 32°C in LBK medium (*14*), buffered at pH 7.6, at a dilution rate of 0.16 hour^{-1}. The section enclosed by the red box is enlarged in the next panel. (**B**) Zoom-in on series 4, 5, and 6, showing variation with time in cell density. The shaded strips represent dilution intermissions.

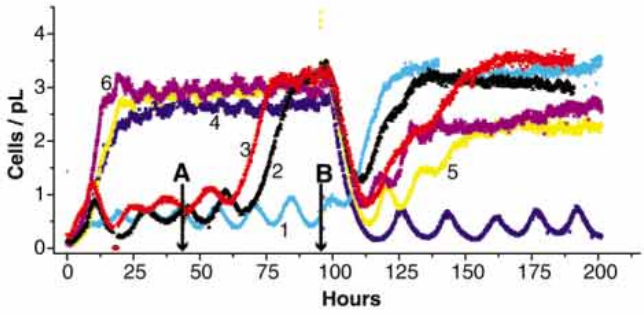

Fig. 4. Growth of Top10F′ cells with the circuit cycling between ON and OFF in the chip. Initially, cultures 1, 2, and 3 were ON, while cultures 4, 5, and 6 were OFF. At 44 hours (point A), cultures 2 and 3 were turned OFF. At 96 hours (point B), culture 1 was turned OFF, and cultures 2 to 6 were turned ON. Cultures 2 and 3 were cultivated on a separate chip in a different experiment under the same conditions. When turned OFF, culture 1 (at 96 hours) and cultures 2 and 3 (at 44 hours) grew exponentially to a density of ~3 cells/pL. Upon circuit activation at 96 hours after an extended OFF period, culture 4 generated sustained oscillations similar to those of culture 1 between 0 and 96 hours, after a rapid decrease in cell density. In comparison, when switched ON at 96 hours, cultures 2, 3, 5, and 6 only briefly demonstrated circuit regulation (evident in the sharp decrease in cell density) before bouncing back to a high density. Cells were grown at 32°C in LBK medium (*14*), buffered at pH 7.0.

Using the ability to monitor individual cells in the microchemostat cultures, we observed that the oscillations in cell density correlated with specific cell morphologies (Fig. 3A). For example, upon inoculation, culture 3 (Fig. 3A, point a) was composed of healthy (small and cylindrical) cells. With negligible expression of the killer protein (LacZα-CcdB) at such low density, the population initially enjoyed exponential growth, in tandem with the OFF cultures. The cells were generally healthy during this phase, evident in their morphology (Fig. 3A, point b). However, as the increased cell density led to increased AHL concentration and, consequently, increased expression of the killer protein (Fig. 3A, point c), the cell density began to decrease. By this time, a fraction of cells had become filamented, showing the deleterious effect of LacZα-CcdB; due to a lag in the turnover of the signal (by dilution and degradation) and that of the killer protein (by cell division and degradation), cell death intensified (Fig. 3A, point d), leading to a sharp decrease in the cell density. Further decreases in cell density ultimately led to a decrease in the signal concentration as well as the killer protein concentration. Eventually, when the death rate dropped below the growth rate as the killer protein was diluted out (Fig. 3A, point e), the population recovered and entered the next cycle. Culture 3 escaped circuit regulation after 186 hours. Under these conditions, the bacterial population oscillated for 4 to 6 cycles before approaching a steady-state concentration of ~2 cells/pL. Cultures 1 and 2 demonstrated similar dynamics.

Different cell morphologies and circuit dynamics were apparent when the population control circuit was introduced into a different *E. coli* host strain. In Top10F′ cells, more complete induction of the circuit was achieved (*14*), leading to stronger growth regulation (Fig. 4). At time 0 in these experiments (with a dilution rate of 0.16 hour^{-1}), the circuit was turned ON in cultures 1 to 3 but left OFF in cultures 4 to 6. The OFF cultures (cultures 4 to 6, 0 to 96 hours) grew to a steady-state density of ~3 cells/pL. In contrast, the ON density was sixfold lower than the OFF density and oscillated about ~0.5 cells/pL (1, 0 to 96 hours; 2 and 3, 0 to 44 hours). Top10F′ cells displayed none of the morphological responses to circuit regulation that were evident in MC4100Z1 cells; they always looked small and cylindrical, similar to circuit-OFF cells.

In general, the circuit appeared to be more stable in the microchemostats than in macroscale batch cultures under otherwise similar growth conditions. During macroscale experiments with reaction volumes of 3 to 50 ml, circuit-ON cultures lost regulation within ~70 hours for MC4100Z1 and 48 hours for Top10F′ cells (*14*). Population control in the microche-

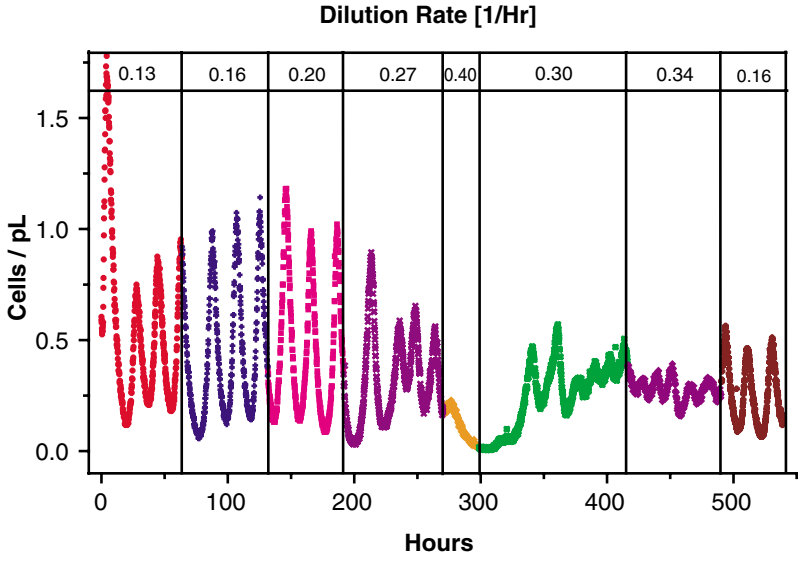

Fig. 5. Effects of the dilution rate on population dynamics of Top10F′ cells with the population-control circuit ON. At high dilution rates (to 0.27, 0.30, and 0.34 hour^{-1}), both the amplitude and the period of oscillations diminished. The culture was approaching wash-out at the highest dilution rate (0.40 hour^{-1}). Large oscillations were recovered when a low dilution rate was restored toward the end of the experiment. Cells were grown at 32°C in LBK medium (*14*), buffered at pH 7.0.

mostat, in contrast, was routinely maintained for more than 200 hours and sometimes more than 500 hours (Fig. 5). In theory, the small population size in the microchemostat (~10^2 to ~10^4 cells versus ~10^9 cells in macroscale cultures) should reduce the overall rate at which mutants may occur and take over a population. Exactly how the cells lost regulation in the circuit-ON cultures (Fig. 3, culture 3, and Fig. 4, cultures 2, 3, 5, and 6) is unclear. A simple mutation rate versus population size argument would predict much longer lifetimes for maintaining circuit regulation.

We observed oscillations in cell density for both *E. coli* strains in the circuit-ON state. The MC4100Z1 strain had large-amplitude (~2 cells/pL) oscillations, which gradually decayed to a steady state (Fig. 3A). The Top10F′ strain had smaller amplitude oscillations (~1 cell/pL) which continued for the length of the measurement (Figs. 4 and 5). Although the origin of these oscillations is unknown, they may be a consequence of the circuit interacting with the continuous culture mechanism of the microchemostat. It is also possible that the oscillations are entirely due to circuit regulation, as predicted by a simple mathematical model with biologically feasible parameters (*14*). These population-level oscillations are controllable—they only occur when the circuit is in the ON state—and they are more sustained and stable than those generated by synthetic oscillators operating in individual cells (*17*, *18*).

An active approach to preventing biofilm formation in microfluidic devices enabled us to implement a miniaturized bioreactor that operates at a working volume of 16 nL, more than 300 times smaller than the smallest previous microfermentor (*7*). This miniaturized device enabled automated culturing and monitoring of populations of ~100 to ~10^4 bacteria with instantaneous single-cell resolution. Reducing the reactor volume by a factor of 10^5 can, in theory, suppress the total mutation rate proportionately and hence prolong monitoring of genetically homogeneous populations (*14*). The microchemostat has enabled us to monitor the programmed behavior of bacterial populations for hundreds of hours despite strong selection pressure to evade population control, something that was not achieved in macroscopic reactors. Although we focused on the bacterial count and cell morphology, measurements can be readily extended to dynamic properties, for example, gene expression dynamics and distributions reported by fluorescence or luminescence. These capabilities enable long-term, low-cost, high-resolution investigation of the phenotypical characteristics of many different cell strains, as well as natural and synthetic cellular networks under a matrix of conditions. This capability will greatly facilitate high-throughput screening applications in fields such as chemical genetics and pharmaceutical discovery.

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tRNA Actively Shuttles Between the Nucleus and Cytosol in Yeast

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Previous evidence suggested that transfer RNAs (tRNAs) cross the nuclear envelope to the cytosol only once after maturing in the nucleus. We now present evidence for nuclear import of tRNAs in yeast. Several export mutants accumulate mature tRNAs in the nucleus even in the absence of transcription. Import requires energy but not the Ran cycle. These results indicate that tRNAs shuttle between the nucleus and cytosol.

Nuclear-encoded tRNAs are transcribed, processed in the nucleus, and exported to the cytosol to facilitate translation (*1*). Moreover, a nuclear pool of mature tRNAs also exists. In *Saccharomyces cerevisiae*, certain mutants defective in nuclear transport and tRNA processing accumulate mature tRNAs in the nucleus, which suggests that nuclear mature tRNAs are intermediates waiting for tRNA export (*2*–*5*). Before export, aminoacyl-tRNA synthetases

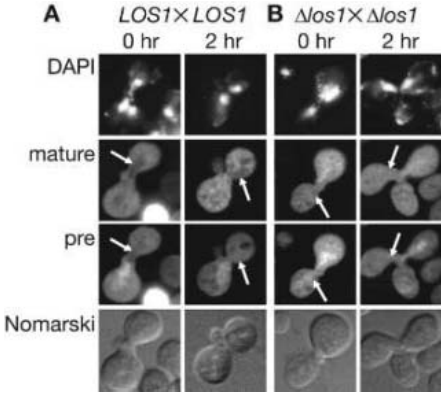

Fig. 1. Heterokaryon assay of nuclear import of tRNAs. (**A**) Heterokaryons formed from *MAT*α cells with *sup3*$^{+}$ and *MAT***a** cells were incubated in yeast extract, peptone, and dextrose (YPD) for the indicated times and subjected to FISH using probes against mature SptRNA-Met$_i$ (mature) and pre-SptRNA-Ser^{UGA} (pre). The nucleus and cell outlines are shown in the 4′,6′-diamidino-2-phenylindole (DAPI) and Nomarski images, respectively. Target nuclei are marked by arrows. (**B**) Heterokaryon assays similar to (A) were performed with Δ*los1* haploids.

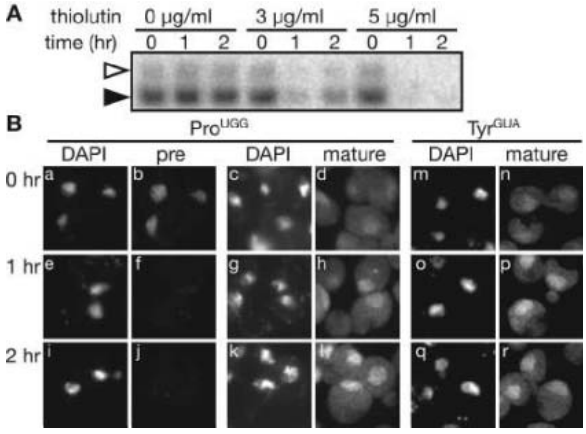

Fig. 2. Treatment with thiolutin causes accumulation of mature tRNA-Pro^{UGG} in Δ*los1* Δ*msn5* cells. (**A**) Δ*los1* Δ*msn5* cells treated with the indicated concentrations of thiolutin were subjected to Northern hybridization with an anti-pre-tRNA-Pro^{UGG} probe. White triangle, primary transcripts; black triangle, end-matured pre-tRNAs. (**B**) The cells treated with 5 μg/ml thiolutin for the indicated times were subjected to FISH with probes against tRNA-Pro^{UGG} and tRNA-Tyr^{GUA}.

may monitor their maturation (*6*). However, we recently found that tRNA-splicing endonuclease localizes to the mitochondrial surface in yeast and that an endonuclease mutant accumulates unspliced pre-tRNAs in the cytosol, which indicates that pre-tRNAs are exported to the cytosol and then spliced (*7*).

To understand the origin of nuclear mature tRNAs, we examined whether tRNAs are imported into the nucleus from the cytosol, using heterokaryon assays (*8*). In *S. cerevisiae*, a heterokaryon with two different nuclei sharing the same cytosol is formed from karyogamy-deficient *MAT***a** and *MAT*α cells. To visualize tRNAs transcribed from one nucleus, a *sup3*$^{+}$ gene (SptRNA-Ser^{UGA}::SptRNA-Met$_i$) from *Schizosaccharomyces pombe* was introduced into the *MAT*α strain. *sup3*$^{+}$ is transcribed and processed to mature SptRNA-Ser^{UGA} and SptRNA-Met$_i$ in *S. cerevisiae* (*9*). Heterokaryons between the *MAT***a** cells (target) and the *MAT*α cells with *sup3*$^{+}$ (donor) were subjected to fluorescence in situ hybridization (FISH). Pre-SptRNA-Ser^{UGA} was detected in only one nucleus in a heterokaryon (Fig. 1A, pre, and fig. S2). In a wild-type background for tRNA export, mature SptRNA-Met$_i$ was excluded from target nuclei like endogenous tRNAs (Fig. 1A and fig. S4B). Δ*los1* cells, lacking yeast exportin-t (*10*, *11*), exhibit a moderate defect in nuclear export of mature tRNAs (fig. S4B) (*2*). In Δ*los1* heterokaryons, we observed more SptRNA-Met$_i$ in target nuclei (Fig. 1B). These data indicate that cytosolic mature tRNAs enter the nucleus.

Next, we investigated nuclear import of endogenous tRNAs in cells growing vegetatively. If all nuclear mature tRNAs are newly transcribed, transcription arrest should reduce the nuclear pool of tRNAs through their export. If mature tRNAs are supplied from the cytosol, this should not necessarily be true. A complicating factor is that the signal intensity of mature tRNAs in the wild-type nucleus is lower than that in the cytosol. To circumvent this problem, we used a Δ*los1* Δ*msn5* double mutant. Msn5p is a homolog of mammalian exportin-5, which exports the tRNA-eEF1A complex and pre-miRNAs (microRNAs) (*12–14*). Δ*msn5* by itself did not alter tRNA localization, but the Δ*los1* Δ*msn5* mutant showed a strong synthetic defect in mature tRNA export (fig. S4), which suggests that Msn5p contributes to this export. Using this mutant, we analyzed the pool of nuclear tRNAs when transcription was blocked by thiolutin, an RNA polymerase inhibitor (*15*). In the presence of thiolutin, pre-tRNA-Pro^{UGG} disappeared within 1 hour (Northern hybridization, Fig. 2A; FISH, Fig. 2B, pre), whereas mature tRNA-Pro^{UGG} and tRNA-Tyr^{GUA} accumulated in the nucleus (Fig. 2B, mature). Because no detectable transcription occurred under these conditions, nuclear mature tRNAs must have been supplied from the preexisting cytosolic pool.

To determine whether nuclear import of mature tRNAs requires energy, Δ*los1* Δ*msn5* cells were incubated with NaN_3 and 2-deoxyglucose (2-dG). Under these conditions, both the primary transcript of tRNA-Pro^{UGG} and the gradient of mature tRNA-Pro^{UGG} across the nuclear envelope (NE) were no longer detected (Fig. 3A and Fig. 3B, e). Reappearance of the primary transcript 10 min after NaN_3 and 2-dG removal indicates rapid replenishment of intracellular adenosine triphosphate (Fig. 3A, –thiolutin). Nuclear accumulation of the mature tRNA was also reestablished after removal of NaN_3 and 2-dG, even in the presence of thiolutin (Fig. 3B, h and k). We observed similar results with tRNA-Ile^{AAU} encoded by intronless genes (Fig. 3B, i and l). These results indicate that

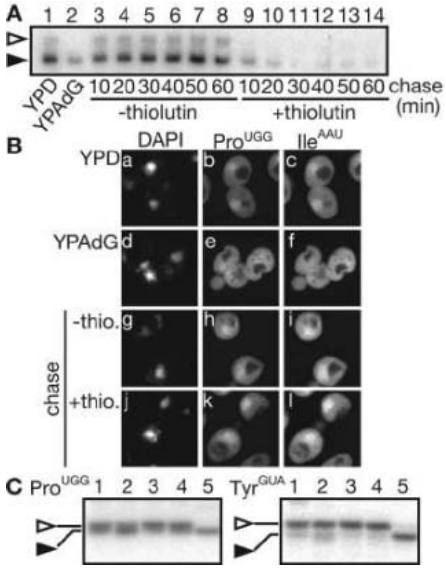

Fig. 3. Nuclear import of tRNAs is energy dependent. (**A**) Δ*los1* Δ*msn5* cells grown in YPD (lane 1) were incubated with NaN_3 and 2-dG (YPAdG) for 1 hour (lane 2). The cells were chased without (lanes 3 to 8) or with (lanes 9 to 14) thiolutin for the indicated times. Pre-tRNA-Pro^{UGG} was detected by Northern hybridization. White triangle, primary transcripts; black triangle, end-matured pre-tRNAs. (**B**) Δ*los1* Δ*msn5* cells were treated as in (A) and were harvested before the energy poison treatment (YPD), after the treatment (YPAdG), and after a 2-hour chase without (–thio.) or with (+thio.) thiolutin. Mature tRNA-Pro^{UGG} and tRNA-Ile^{AAU} were visualized with FISH. (**C**) Δ*los1* Δ*msn5* cells grown in YPD (lane 1) were incubated in YPAdG (lane 2) and chased with thiolutin for 1 hour (lane 3) or 2 hours (lane 4). Aminoacylation states of tRNA-Pro^{UGG} and tRNA-Tyr^{GUA} were analyzed with acidic urea-PAGE. Lane 5 contains deacylated tRNA. White and black triangles represent aminoacylated and deacylated tRNAs, respectively.

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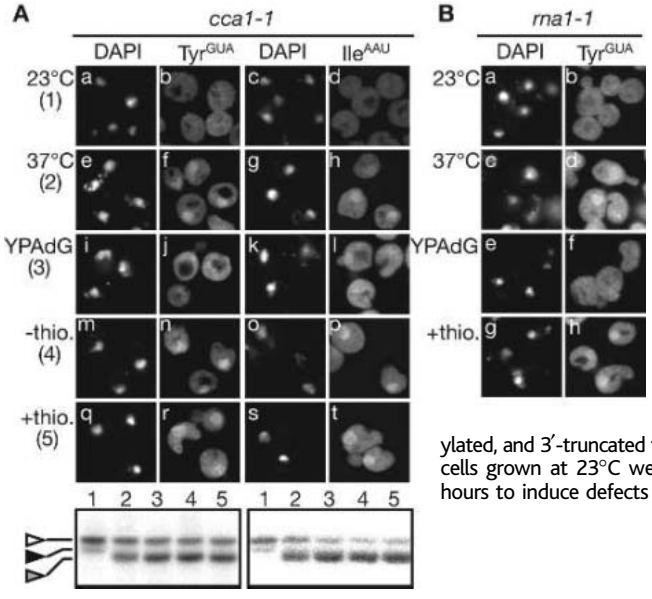

Fig. 4. Various tRNA species are imported into the nucleus, and import is Ran independent. (**A**) *cca1-1* cells grown at 23°C were incubated at 37°C for 3 hours, treated with NaN_3 and 2-dG for 3 hours at 37°C (YPAdG), and then chased without (–thio.) or with (+thio.) thiolutin for 1.5 hours. Samples were analyzed using FISH (top) and Northern hybridization (bottom) with indicated probes. White, black, and gray triangles represent aminoacylated, deacylated, and 3′-truncated tRNAs, respectively. (**B**) *rna1-1* cells grown at 23°C were incubated at 37°C for 1.5 hours to induce defects and then processed as in (A).

the import of both intron-containing and intronless tRNAs requires energy.

We analyzed aminoacylation states of mature tRNAs imported into the nucleus using acidic urea–polyacrylamide gel electrophoresis (urea-PAGE). Mature tRNA-Pro^{UGG} and tRNA-Tyr^{GUA} in Δ*los1* Δ*msn5* cells were present primarily as slower migrating forms even after the chase with thiolutin (Fig. 3C, lanes 1, 3, and 4). These forms were converted to faster migrating forms by base treatment (lane 5), which indicates that the slower migrating tRNAs are aminoacylated. Therefore, imported tRNAs exist mainly in aminoacylated forms in the nucleus.

We asked whether 3′-truncated tRNAs are imported. *cca1-1* cells are deficient in both de novo formation and repair of 3′-terminal CCA ends of tRNAs (*16*), and also in tRNA export (*4*). We therefore treated *cca1-1* cells with NaN_3 and 2-dG at a restrictive temperature to abolish the mature tRNA gradient across the NE and chased them with thiolutin. tRNA-Tyr^{GUA} and tRNA-Ile^{AAU} reaccumulated in the nucleus to similar levels (Fig. 4A, q to t), although the proportions of the 3′-truncated forms of these tRNAs were different (Fig. 4A, bottom). Taken together with the fact that tRNAs in Δ*los1* Δ*msn5* cells were full-length tRNAs, these results indicate that various forms of tRNAs are imported.

To determine the contribution of Ran guanosine triphosphatase (GTPase) (*17*), we examined tRNA import in *rna1-1* cells defective in RanGAP (Ran GTPase activating protein). The *rna1-1* cells accumulated mature tRNA-Tyr^{GUA} in the nucleus at 37°C (*2*), and this tRNA gradient disappeared upon treatment with NaN_3 and 2-dG (Fig. 4B, 37°C, YPAdG). When the cells were transferred to medium with thiolutin, mature tRNA-Tyr^{GUA} was reaccumulated (Fig. 4B, +thio.). Because protein import ceased at 37°C in *rna1-1* cells (fig. S5), these results suggest that tRNA import is Ran independent.

Our results indicate that nuclear mature tRNAs are supplied from the cytosol and that tRNAs shuttle between these two compartments. Shuttling may contribute to tRNA quality control, because tRNAs have long lifetimes and may run the risk of inappropriate modifications (*18*). A quality-control system in the nucleus may repair or filter out inactive tRNAs from those shuttling through the nucleus and provide only active tRNAs back to the cytosol. Another controversial possibility would be to supply tRNAs for nuclear translation (*19*).

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Variable Control of Ets-1 DNA Binding by Multiple Phosphates in an Unstructured Region

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Cell signaling that culminates in posttranslational modifications directs protein activity. Here we report how multiple Ca^{2+}-dependent phosphorylation sites within the transcription activator Ets-1 act additively to produce graded DNA binding affinity. Nuclear magnetic resonance spectroscopic analyses show that phosphorylation shifts Ets-1 from a dynamic conformation poised to bind DNA to a well-folded inhibited state. These phosphates lie in an unstructured flexible region that functions as the allosteric effector of autoinhibition. Variable phosphorylation thus serves as a "rheostat" for cell signaling to fine-tune transcription at the level of DNA binding.

Proteins are activated or repressed by posttranslational modifications in response to extracellular cues. Phosphorylation, a typical modification, often accumulates at multiple sites until a threshold level is reached. The outcome is described as a sharp on/off switch of protein activity (*1*, *2*). However, biological processes may need more sensitive regulation. Despite this need, variable regulation of protein activity in response to multiple modifica-

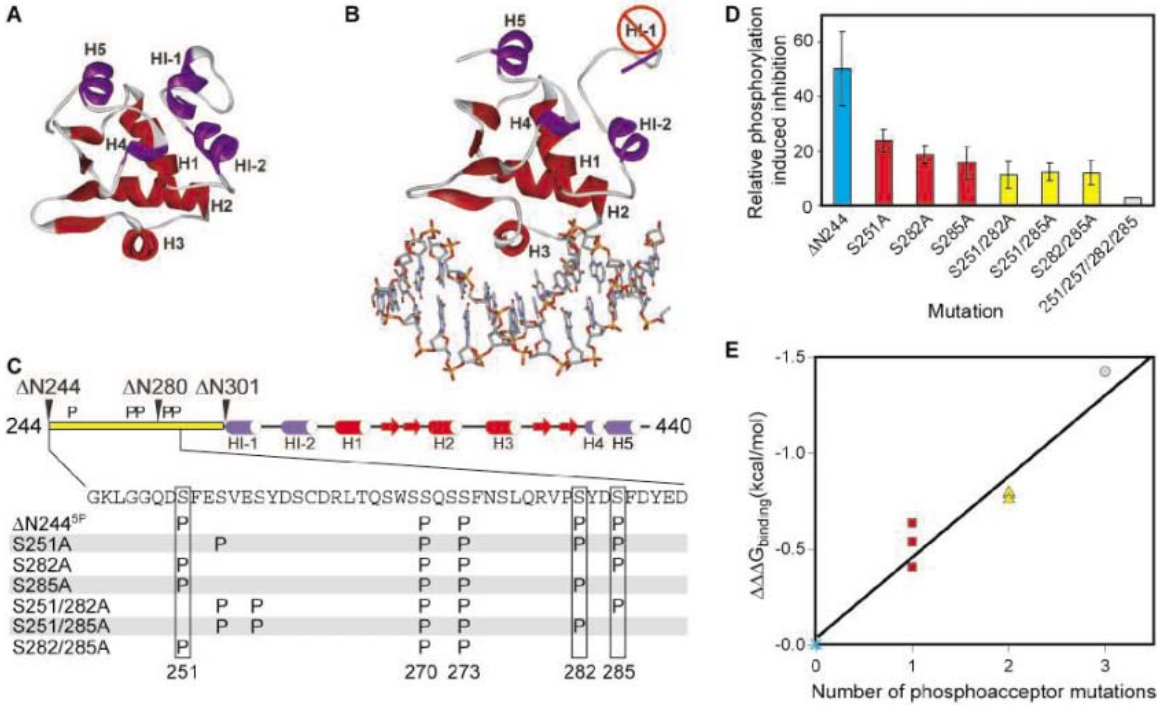

Fig. 1. Differential phosphorylation of Ets-1 leads to variable DNA binding affinity. (**A**) NMR-derived structure of the partially active Ets-1 truncation ΔN301, showing the ETS domain (red) and inhibitory helices (purple) (*8*). (**B**) Crystallographic structure of DNA-bound ΔN280, showing inhibitory helix HI-1 unfolded (*31*). (**C**) Schematic secondary structure of ΔN244 with SRR (yellow) and sites phosphorylated by CaMKII in vitro (labeled P) as determined by mass spectrometry and/or NMR for the wild type and mutants. Boxed phosphoserines are critical for the reinforcement of autoinhibition. Additional phosphates have negligible individual effects and vary with mutation pattern (*32*). (**D**) Relative phosphorylation-induced inhibition = $K_{D(\text{phosphorylated mutant})}/K_{D(\text{unmodified mutant})}$ with the standard error of the ratio ($n \geq 4$ replicas) (*15*). Single mutants are colored red and double mutants are colored yellow. The quadruple mutant (gray) (*11*) provides expected data for the disruption of three critical phosphoacceptor sites (251, 282, and 285). (**E**) $\Delta\Delta\Delta G^{o}_{binding} = -RT$ ln(relative induced inhibition, ΔN244) + RT ln(relative induced inhibition, mutant), where R is the ideal gas constant and T is temperature, is plotted as a function of the number of phosphoacceptor mutations (wild type, exes; single, squares; double, triangles; triple, circle). The linear relationship (correlation coefficient r = 0.96) implicates additivity of mutational effects (*33*).

tions has not been documented. Here we show how the transcription factor Ets-1 exhibits graded DNA binding activity with different levels of phosphorylation.

Ets-1 is regulated by an autoinhibitory module composed of four α helices (HI-1, HI-2, H4, and H5) that flank the DNA-binding ETS domain. In the native protein, these helices pack cooperatively on a surface of the ETS domain that is opposite to the DNA binding interface (Fig. 1A), reducing the affinity of Ets-1 for DNA 10-fold, compared with the affinity of the minimal ETS domain (*3–5*). This inhibitory module opposes the structural change that accompanies DNA binding, the most striking feature of which is the unfolding of inhibitory helix HI-1 (Fig. 1B) (*3*, *6–8*). Recent nuclear magnetic resonance (NMR) spectroscopic studies indicate that helix HI-1 is labile, even in the absence of DNA, and could serve as a control point for modulating DNA binding affinity (*8*). Cooperative DNA binding with runt-related protein 1 (RUNX1) counteracts autoinhibition, activating the binding of native Ets-1 ~10-fold (*9*), whereas Ca^{2+}-dependent phosphorylation of Ets-1 at multiple sites reinforces autoinhibition by lowering DNA affinity ~50-fold further to an overall inhibition of 500- to 1000-fold (fig. S1, A to D) (*10*, *11*). The modulation of Ets-1 DNA binding is correlated with transcriptional activity in in vivo assays and is responsive to cellular cues (*12–14*). For example, Ca^{2+} signaling in vivo disrupts Ets-1 DNA binding and reduces the transcription of Ets-1–driven reporters (*13*). We show that this multiple phosphorylation is not simply an on/off switch, but rather an incremental rheostat-like control for Ets-1 binding activity.

We used a minimal fragment, ΔN244, which displays the DNA binding properties of full-length Ets-1 (fig. S1, A to D) (*11*, *15*). $\Delta N244^{5P}$, a form which is homogenously phosphorylated by CaMKII, contains five specific phosphoserines in the serine-rich region (SRR, residues 244 to 300) (Fig. 1C) and appears to undergo the same structural transition upon DNA binding as do unmodified ΔN244 (fig. S1E) and other Ets-1 species (*3*, *7*, *11*).

In cells, Ets-1 is variably phosphorylated in response to Ca^{2+} release (*10*). To gauge the effect of differential phosphorylation on activity, we mimicked in vivo phosphorylation by mutating phosphoacceptor serines to alanines singly and in pairs (Fig. 1C), and we then measured the DNA binding affinity of phosphorylated and unmodified forms. Mutation of three (251, 282, and 285) of the five serines, but not two others (270 and 273), significantly reduced inhibition. Furthermore, mutation of one, two, or all three critical sites (*11*) yielded a graded reduction in phosphorylation-dependent inhibition (Fig. 1D), with each phosphate contributing additively to the change in the free energy of binding (Fig. 1E). This finding shows that the number and context of sites, and not simply a buildup of charge, affects inhibition. Thus, in contrast to proteins such as Sic1 (*2*) and NFAT1 (nuclear factor of activated T cells) (*1*, *16*), for which a threshold level of phosphorylation serves as a binary switch, multiple sites within Ets-1 regulate DNA binding in a graded manner across a wide range of affinities, which is consistent with observed variable regulation in vivo (*13*).

The phosphorylated SRR was found to be predominantly unstructured and highly flexible. Based on main-chain ^{1}H and ^{13}C chemical shifts, NMR spectra revealed no predominant secondary structure within this region (*17*) (Fig. 2A), and the amide hydrogen exchange (HX) rates were comparable to those of a random coil polypeptide (Fig. 2B). Further, NMR relaxation experiments demonstrated a high degree of backbone conformational mobility on nanosecond to picosecond time scales (Fig. 2C). Thus, the dynamic phosphorylated SRR may impart variable regulation of DNA binding by making

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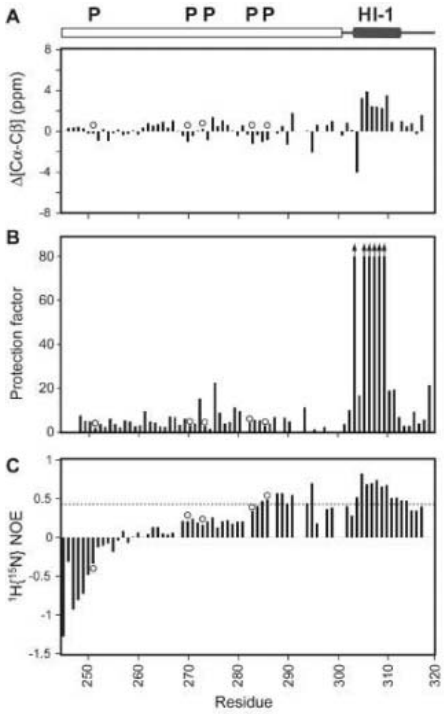

Fig. 2. The phosphorylated SRR is predominantly unstructured and flexible. Panels present data for residues 244 to 320 of $\Delta N244^{5P}$ (*15*). Circles and P denote the five phosphoserines. Missing data correspond to prolines, or residues with spectral overlap and/or weak signals. (**A**) In contrast to HI-1, differences between the observed and random coil ($^{13}C^{\alpha}$-$^{13}C^{\beta}$) chemical shifts indicated an absence of any detectable secondary structure in the SRR (*17*). (**B**) Small protection factors (k_{pred}/k_{ex}) demonstrate that backbone SRR amides undergo HX at rates (k_{ex}) similar to those predicted for a random coil polypeptide (k_{pred}). Amides within HI-1 do not show measurable HX by the CLEANEX method ($k_{ex} < 0.5\ s^{-1}$, protection factors > 80). (**C**) Small or negative heteronuclear ^{1}H{^{15}N} nuclear Overhauser enhancement values indicate a high degree of flexibility. The horizontal line at 0.4 marks an approximate boundary between flexible (below) and rigid (above) amides on a subnanosecond time scale (*34*).

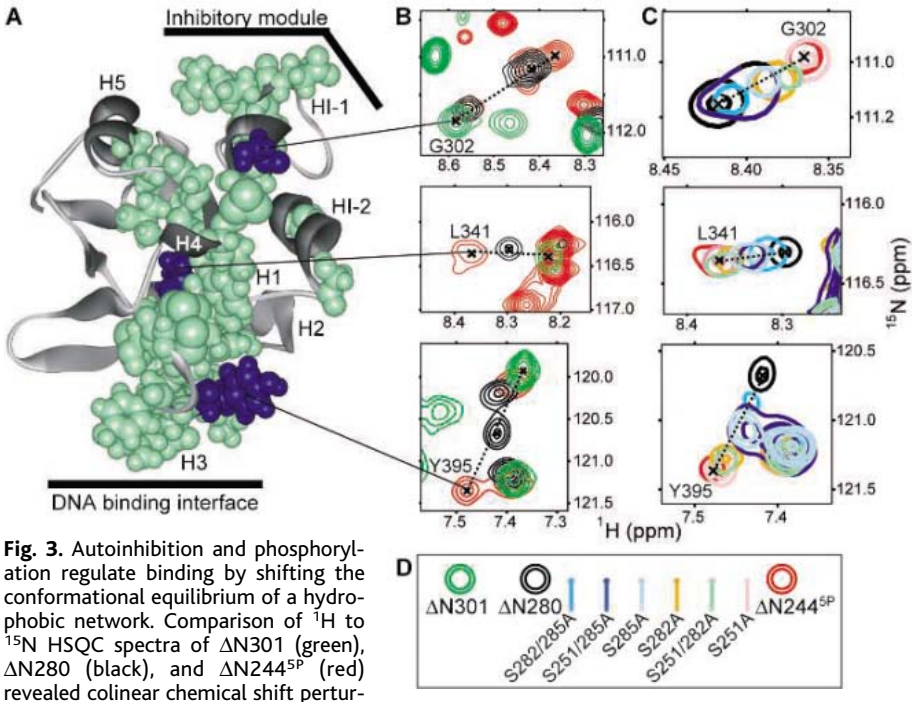

Fig. 3. Autoinhibition and phosphorylation regulate binding by shifting the conformational equilibrium of a hydrophobic network. Comparison of ^{1}H to ^{15}N HSQC spectra of ΔN301 (green), ΔN280 (black), and $\Delta N244^{5P}$ (red) revealed colinear chemical shift perturbations among ~25 common residues in the three species (table S1 and fig. S3) (*15*). (**A**) Shifted residues depicted on the NMR-derived structure of ΔN301 by van der Waals surfaces (*8*). (**B**) Spectral overlays highlight the progressive shift changes (black exes and dashed lines) for G302, L341, and Y395 (*20*). (**C**) Peaks from six phosphorylated phosphoacceptor mutants follow the same colinear chemical shift pattern (between ΔN280 and $\Delta N244^{5P}$ peaks on dashed lines). (**D**) Color key and consensus relative peak positions.

transient interactions with the remainder of the protein.

Despite this flexibility, the SRR has a profound impact on the structure, stability, and dynamics of the DNA binding domain and on the inhibitory helices. We compared three Ets-1 fragments with increasing levels of inhibition: partially activated ΔN301; ΔN280, a minimal fragment that recapitulates unmodified autoinhibited Ets-1 binding; and $\Delta N244^{5P}$ (dissociation constants $K_D \sim 10^{-11}$, 10^{-10}, and 10^{-8} M, respectively) (fig. S1, A, B, and D) (*6*, *8*, *18*). The comparison of ^{1}H-^{15}N heteronuclear single quantum correlation (HSQC) spectra detected significant changes in backbone amide chemical shifts for ~25 residues common to the three species, indicative of structural perturbations (figs. S2 and S3 and table S1). The labile inhibitory helix HI-1 and helices H1 and H3 of the DNA binding domain were most widely affected. The perturbed residues, many of which are nonpolar, form a hydrophobic network connecting the inhibitory elements and the DNA binding interface (Fig. 3A). Mutation of leucine-429 to alanine within this network reduced autoinhibition and impaired phosphorylation reinforcement (fig. S1C) (*5*, *11*), indicating that the integrity of this hydrophobic network is required for regulation.

A striking linear pattern of change was observed from ΔN301 to ΔN280 to $\Delta N244^{5P}$ in the amide ^{1}H and ^{15}N chemical shifts of almost all of the affected residues (Fig. 3B and table S1). This progressive colinear pattern is a signature of an allosterically regulated molecule that is in conformational equilibrium between at least two states, with the intermediate chemical shifts representing a population-weighted average of these states (*19*). Based on the correlation between the linear pattern of amide chemical shifts with DNA binding affinity (*20*), we propose that free Ets-1 exists in equilibrium between an active state (represented most closely by ΔN301), which is poised to bind DNA, and an inactive state (represented by $\Delta N244^{5P}$). According to this allosteric model, Ets-1 DNA binding affinity reflects the balance of these two states.

A comparison of the backbone amide chemical shifts of phosphorylated ΔN244 with single or double serine-to-alanine mutations also revealed a colinear shifting pattern between ΔN280 and $\Delta N244^{5P}$ in at least 16 of the 25 residues within the aforementioned hydrophobic network. The position of the chemical shifts for each species, phosphorylated at the remaining CaMKII sites, roughly correlated with affinity. This finding emphasizes the context dependence of each phosphate and suggests that each has a distinct role in modulating the conformational equilibrium (Fig. 3, C and D, and table S1). These NMR studies show that differential phosphorylation regulates DNA binding by fine-tuning the balance between the active and inactive states of Ets-1.

Amide HX experiments helped characterize the dynamics of the active and inactive states (Fig. 4, A to C, and fig. S4). Consistent with previous studies (*8*), backbone amides within the inhibitory helices HI-1 and HI-2, as well as the DNA binding helix H3 of the active ΔN301, exhibited limited protection from exchange, indicating that these helices sample locally unfolded conformations even in the absence of DNA. In contrast, the same amides exhibited elevated HX protection within ΔN280, and further in $\Delta N244^{5P}$, suggestive of a progressively less dynamic and more stably folded species. The dynamic active state and more stable inactive state are consistent with previous studies, showing that affinity for DNA decreases as the propensity of helix HI-1 to unfold decreases (*3*, *7*, *11*).

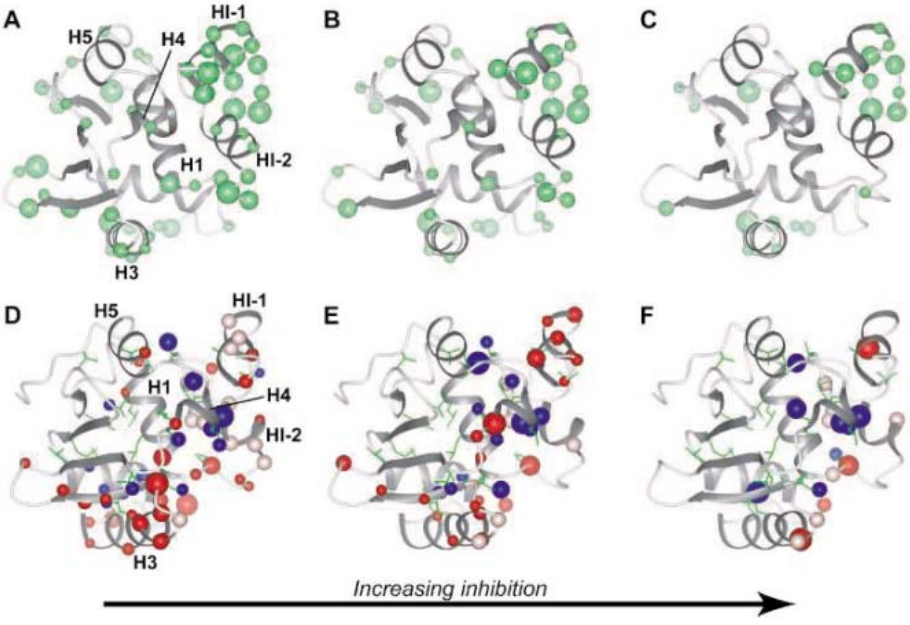

Fig. 4. Concerted motion of the hydrophobic network is dampened by autoinhibition and phosphorylation. ΔN301 (**A** and **D**), ΔN280 (**B** and **E**), and ΔN244^{5P} (**C** and **F**) were analyzed by amide HX and backbone and side-chain relaxation dispersion (*15*). Relative values (figs. S4 to S6) are highlighted on the NMR-derived structure of ΔN301 (*8*). [(A), (B), and (C)] For backbone HX, amides exhibiting measurable exchange (k_{ex} > 0.5 s^{-1}) are depicted by green balls with diameters scaled according to increasing k_{ex}. [(D), (E), and (F)] For relaxation dispersion, backbone and Trp side-chain nitrogens (red balls) and ^{13}C methyls of Ile$^{\delta 1}$, Val, and Leu (blue balls) with $R_{ex} \geq 2$ s^{-1} are shown, where the diameter of the balls increases in proportion to R_{ex} at 800 MHz (the largest balls correspond to $R_{ex} \geq$ 15 s^{-1}). Pink balls represent atoms whose NMR signals are broadened beyond detection. Ile, Leu, and Val side chains are shown as green sticks.

A dynamic connection between the autoinhibitory module and the DNA binding interface was demonstrated by NMR backbone amide and side-chain methyl relaxation dispersion measurements (Fig. 4, D to F, and figs. S5 and S6) (*21*). The contribution of millisecond to microsecond time scale motions to linewidth broadening, denoted R_{ex}, is indicative of conformational switching. Consistent with previous studies (*8*), the backbone amides of ΔN301 exhibited mobility on this time scale (exchange lifetimes τ_{ex} ~ 0.3 ms) for residues that shift colinearly between ΔN301, ΔN280, and ΔN244^{5P}. These motions were dampened in ΔN280, and further in ΔN244^{5P} (Fig. 4, red balls). ^{13}C methyl relaxation dispersion of Leu, Ile, and Val side chains within the hydrophobic network of ΔN301 showed motions on the same time scale (τ_{ex} ~ 0.3 ms) that again were progressively reduced in ΔN280 and ΔN244^{5P} (Fig. 4, blue balls). These data indicate that the core hydrophobic packing of Ets-1 is dynamic in the active state and that increasing inhibition attenuates these motions. Localized collective motions on a similar time scale within both the backbone and side chains support our proposal that the inhibitory module, DNA binding interface, and hydrophobic core form a concerted unit that is linked as a dynamic hydrophobic network. Furthermore, the HX and R_{ex} data are consistent with a conformational equilibrium within this concerted unit between at least two states: one stable and inactive, another more dynamic and active.

The dynamic character of Ets-1 is likely essential for sequence-specific DNA binding but also provides an opportunity for regulation (*22*, *23*). NMR studies of the *Escherichia coli* lactose repressor protein suggest that fluctuations on a millisecond to microsecond time scale are necessary for facilitated diffusion on nonspecific DNA, as well as facile adoption of a specifically bound conformation (*24*). The active state of Ets-1 is similarly poised to adopt a high-affinity interaction with DNA. In this case, signal-dependent phosphorylation employs these motions to vary activity in a graded manner.

The surprising use of a highly flexible segment in the graded regulation of Ets-1 DNA binding adds to the growing recognition of the role of unstructured protein regions in biology (*25*). These flexible elements often display posttranslational modifications [for example, MAPK Ets-1 phosphorylation (*26*), histone tails (*27*), and SH2 domain targets (*28*)], tether independent domains [for example, protein kinase A (PKA) (*29*)], or require folding for activity [for example, the cyclic adenosine 3′,5′-monophosphate response element binding protein (CREB) and the CREB binding protein (*30*)]. The highly mobile SRR demonstrates a role for unstructured protein segments in integrating signals that direct the variable regulation of protein activity.

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Effects of Landscape Corridors on Seed Dispersal by Birds

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Habitat fragmentation threatens biodiversity by disrupting dispersal. The mechanisms and consequences of this disruption are controversial, primarily because most organisms are difficult to track. We examined the effect of habitat corridors on long-distance dispersal of seeds by birds, and tested whether small-scale (<20 meters) movements of birds could be scaled up to predict dispersal of seeds across hundreds of meters in eight experimentally fragmented landscapes. A simulation model accurately predicted the observed pattern of seed rain and revealed that corridors functioned through edge-following behavior of birds. Our study shows how models based on easily observed behaviors can be scaled up to predict landscape-level processes.

Habitat fragmentation poses a widespread threat to biodiversity by disrupting the dispersal of organisms (*1*, *2*). Corridors—narrow strips of habitat that join patches of similar habitat—are thought to provide a general solution by restoring dispersal among patches, thereby increasing gene flow and reducing the probability of local extinctions (*3*, *4*). Yet corridors are controversial (*5*, *6*). Their efficacy can vary greatly among systems, depending on the complex interaction between disperser behavior and landscape structure (*3*, *7*). Controversy about corridors has been difficult to resolve because corridors operate at a landscape scale, where both experimental and observational (tracking) studies are difficult (*8*).

Here we test and validate an alternative approach to examine corridor function: individual-based behavioral models (*9*). Our goal was to predict corridor effects on long-distance (>250 m) dispersal of seeds by birds. We collected data on small-scale (<20 m) movements of seed-dispersing birds in experimental landscapes and used these movements to parameterize a model that predicted the effects of corridors on seed dispersal at the landscape scale. We then tested our model using data on actual seed rain from the same large-scale experimental landscapes. Importantly, our model links observations of local bird behavior to population-level impacts on the recruitment of plants, showing how corridor effects on one taxon can affect the other. More generally, the control and replication provided by our experimental landscapes allow a rigorous validation of individual-based models, which are widely applicable to other systems.

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Our experimental landscapes were designed to test two alternative hypotheses about how corridors function. The traditional corridor hypothesis posits that corridors act as dispersal conduits, channeling organisms between connected patches (*3*, *4*). The drift-fence hypothesis posits that corridors intercept organisms dispersing through matrix habitat and direct them into associated patches, thereby increasing colonization of patches with corridors, regardless of whether the corridors connect patches (*10*, *11*). These hypotheses are not mutually exclusive.

Each of our experimental landscapes contained five patches of regenerating vegetation in a matrix of mature pine forest (Fig. 1) (*12*). A central "source" patch (100 m by 100 m) was separated by 150 m from four peripheral "receiver" patches. One receiver patch in each landscape was connected to the source patch by a 25-m-wide corridor. Another receiver patch had two 25 m by 75 m corridors ("wings") extending from opposite sides of the patch, perpendicular to the direction of organisms dispersing from the source patch, but not connected to any other patch. A third type of receiver patch was rectangular (100 m by 137.5 m).

Because the areas of winged and rectangular patches were equal to the summed area of the connected patch and its corridor, we could test for corridor effects while controlling for area. The traditional corridor hypothesis predicts that seed dispersal from the source patch into the connected receiver patch will be greater than dispersal into unconnected receiver patches. The drift-fence hypothesis predicts that dispersal into winged patches will be higher than dispersal into rectangular patches, because the cross-sectional area of winged patches from the perspective of an organism in the source patch is greater than that of rectangular patches.

Our results are most directly applicable to savannah (historically, the habitat at our site), prairie, or other open habitats. However, our system also provides a general model for testing corridor theory, applicable to any fragmented landscape in which patches and corridors of suitable habitat are surrounded by a matrix of unsuitable habitat (e.g., patches of mature habitat in matrices of disturbed habitat).

Our study species were wax myrtle (*Myrica cerifera*) and one of its major seed dispersers in South Carolina, Eastern Bluebirds (*Sialia sialis*). Both species generally prefer open habitat. We placed fruiting wax myrtle bushes in the central patch of each landscape. Each time bluebirds were observed eating fruit from these bushes, we tracked their movements with a team of three people, using voice-activated radios to coordinate observations. Trackers stayed as far away as possible (>25 m), taking care not to influence the bird's direction of travel. Bluebirds did not seem to be affected by human presence (*13*). To increase independence of observations, we generally restricted ourselves to tracking one bird per experimental landscape per day. Bluebirds are not territorial in the winter, when wax myrtle bears fruit.

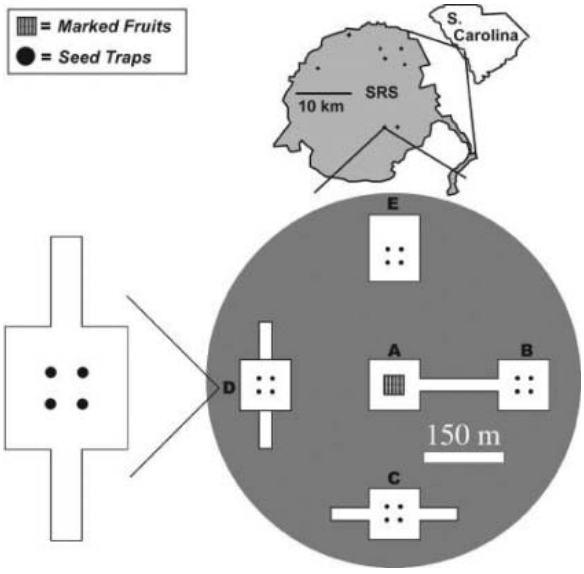

Fig. 1. One of eight experimental landscapes (black dots on map of SRS, the Savannah River Site National Environmental Research Park), showing the four patch types. Each experimental landscape had a source patch (A), where marked fruits were placed in the patch's center, and three types of receiver patches, where seed traps were placed. One receiver patch in each landscape was attached to the source patch by a corridor (B). In four landscapes two of the remaining three receiver patches were winged (C and D) and one was rectangular (E; as pictured here), and in the other four landscapes, two receiver patches were rectangular and one was winged.

Edges strongly influenced movement directions (χ^2 = 48.9, df = 15, P < 0.001; moves combined into 16 groups of 22.5°) (Fig. 2) (*13*). When a bird encountered an edge, it was most likely to fly parallel to it (0° or 180°) or less often, directly perpendicular, across or away from it (90° or 270°). We used these and other tracking data to estimate distributions of move direction, move length, and perch time (time between moves) as a function of habitat, distance to edge, and the orientation of the nearest edge and previous move (*13*). The median number of perch locations was 10 (1st and 3rd quartiles = 6 and 14), and median flight distance between perches was 17 m (1st and 3rd quartiles = 11 and 29 m).

To predict where seeds ingested by bluebirds in the source patch would be defecated, we simulated bluebird movement based on the fitted models, starting with the bird at the center of the source patch and ending after 45 min of movement (an estimate of seed retention time in bluebirds) (*13*). We calculated the proportion of simulated individuals located within the center 25 m by 25 m of receiver patches at the end of one simulation in each experimental landscape (n = 8 total), where one simulation consisted of 20,000 dispersal events. We used the center 25 m by 25 m of patches for simulation results because we had collected empirical data on dispersed seeds in this area (see below), thereby allowing a perfect match between the scales at which we modeled and measured seed dispersal. From the perspective of most plants, this constitutes movement at the landscape scale, because most vertebrate-dispersed seeds travel <50 m (*14*).

The distribution of birds at the end of 45 min was highly nonrandom ($F_{1,29}$ = 79.2, P < 0.001). Birds were 31% more likely to be found in the center of connected patches than the center of unconnected winged and rectangular patches (Fig. 3). The percentage of birds in the two types of unconnected patch types did not differ ($F_{1,29}$ = 1.97, P = 0.17). These results support the traditional corridor hypothesis and not the drift-fence hypothesis.

To independently test model predictions, we tracked the movement of individual seeds from wax myrtle plants in the source patches to seed traps suspended from the tops of poles in the center of all receiver patches (Fig. 1). Bluebirds were responsible for depositing most seeds found in the traps, because they accounted for 79% of observations of fruit-eating birds perched above traps (n = 90). We tracked seeds from wax myrtle planted in the central patches to our traps in receiver patches by spraying their fruits with a dilute solution of fluorescent powder and examining defecations for fluorescence (*15*). Defecated seeds without fluorescent powder were eliminated from analyses. We report data from two field seasons.

The model accurately predicted the observed distribution of wax myrtle seeds collected from >11,000 defecations in seed traps (Fig. 3). On average, seeds in traps were 37% more likely to be found in the center of connected patches than in the center of unconnected winged and rectangular patches, thereby supporting the traditional corridor hypothesis ($F_{1,60}$ = 20.64, P < 0.0005). Winged and rectangular patches received similar proportions of seed rain ($F_{1,60}$ = 0.13, P = 0.724), leading us to reject the drift-fence hypothesis. These patterns did not differ between years ($F_{1,60}$ = 0.62, P = 0.804).

These results have conservation relevance. Land managers must frequently decide whether to allocate limited resources to improving connectivity versus alternatives such as acquiring unconnected land. Our results provide a landscape-level demonstration that habitat corridors substantially increase the movement of birds and seeds between connected patches of habitat. Given that all receiver patches were equal in area, we conclude that the benefits of corridors extend beyond the increased amount of habitat they provide. Also, the benefits of corridors clearly apply more broadly than typically presumed. In this case, plant populations prosper through their interactions with animal mutualists. Although we studied dispersal of a common species, bluebirds disperse seeds of many species, including some of management concern. Our results extend to these species because of their shared dispersal agent.

Models are most useful when they yield unexpected insights that become obvious in hindsight. Because bluebirds tended to follow edges, the corridor effect we observed was not due to the corridor per se, but rather to its edge. Indeed, we never witnessed a bluebird traveling between patches through a corridor; they always did so through the matrix, traveling alongside the corridor. Because all corridors have edges, this mechanism has broad implications. First, it shows that the functional connectivity of corridors extends beyond the structural connectivity they provide (*7*, *16*). This complicates the evaluation of corridors—contrary to common sense, lack of travel in a corridor and frequent dispersal through the matrix do not necessarily contradict the value of corridors in maintaining habitat connectivity. Second, corridor width and other attributes of corridor quality may be irrelevant to organisms that behave like bluebirds—for such organisms, the defining attribute of a corridor is its edge. It follows that different types of edges may influence landscape connectivity in much the same way that different types of matrices do (*17*–*20*). The emerging lesson is that corridor effects and edge effects are intertwined; understanding how organisms respond to edges is central to understanding how corridors function (*21*, *22*).

More generally, our results show how local behaviors can be scaled up to reveal larger-scale patterns of dispersal. In our system, flights that were typically <20 m were used to successfully predict long-distance seed dispersal that occurred over hundreds of meters. The importance of understanding how landscape structure affects the behavior of dispersing organisms is a common theme in landscape ecology (*1*, *20*, *23*–*27*), yet following animals across large landscapes for time periods long enough to characterize dispersal and colonization is nearly impossible. This problem is exacerbated by co-occurrence of corridors with waterways, roads, and other uncontrolled features of natural landscapes, preventing causal inference about corridor effects. The most common solution is to model how individuals react to habitat features, then extrap-

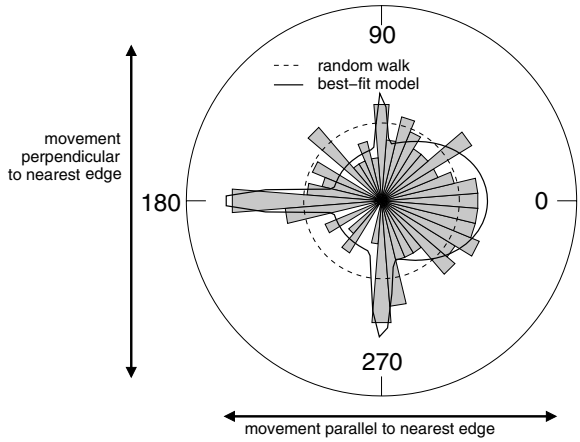

Fig. 2. Distribution of movement directions of bluebirds located in the forest and close to a single edge. Gray wedges show observed data; solid line shows the best-fit movement model. All directions are relative to the consistent direction of travel—a vector parallel to the nearest edge, oriented in the same 180° arc as the previous move direction (supporting online text).

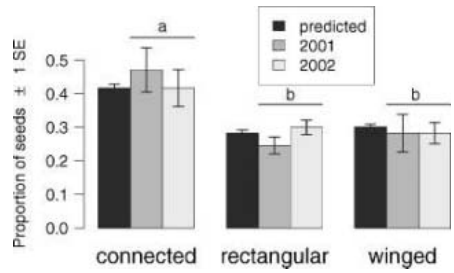

Fig. 3. Predicted distribution of seeds among receiver patches, based on simulated movements of bluebirds within experimental landscapes ("predicted"), and observed distributions of wax myrtle seeds captured in seed traps for two field seasons ("2001" and "2002"). Error bars denote SE and matching letters indicate nonsignificant differences between their corresponding patch types.

olate dispersal behavior to the landscape level (*17*, *21*, *27*, *28*). Explicit tests of such models are needed (*18*). The tight fit between observed and predicted patterns of seed rain in our habitat patches provides strong support for the key assumption that small-scale behavioral responses can drive landscape-scale distributional patterns. From a conservation perspective, impacts of corridors can be predicted on the basis of behaviors that are relatively simple to measure (*29*).

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Identification of a Universal Group B *Streptococcus* Vaccine by Multiple Genome Screen

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Group B *Streptococcus* (GBS) is a multiserotype bacterial pathogen representing a major cause of life-threatening infections in newborns. To develop a broadly protective vaccine, we analyzed the genome sequences of eight GBS isolates and cloned and tested 312 surface proteins as vaccines. Four proteins elicited protection in mice, and their combination proved highly protective against a large panel of strains, including all circulating serotypes. Protection also correlated with antigen accessibility on the bacterial surface and with the induction of opsonophagocytic antibodies. Multigenome analysis and screening described here represent a powerful strategy for identifying potential vaccine candidates against highly variable pathogens.

Group B *Streptococcus* (GBS) is the foremost cause of life-threatening bacterial infections in newborns (*1*). In about 80% of cases, neonatal GBS infection is acquired during delivery by direct mother-to-baby transmission of the pathogen, which colonizes the anogenital mucosa of 25 to 40% of healthy women (*2*). Despite the introduction of intrapartum antibiotic prophylaxis, in the United States GBS still causes ~2500 cases of infection and 100 deaths annually among newborns in the first 3 months of life (*3*). About half of these cases occur in the first week after birth. Thus, it is commonly believed that effective vaccination will be the only way to reduce the incidence of GBS disease over the long term. The rationale for GBS vaccine development is supported by the observation that the risk of neonatal infection is inversely proportional to the maternal amounts of specific antibodies to the capsular polysaccharide (CPS) antigen that surrounds GBS (*4*, *5*), the implication being that protective immunoglobulin G (IgG) antibodies are transferred from the mother to the baby through the placenta.

As a first approach to vaccine development, CPS-tetanus toxoid conjugates against all nine GBS serotypes were shown to induce CPS-specific IgG that is functionally active against GBS of the homologous serotype (*6*). Clinical phase 1 and phase 2 trials of conjugate vaccines prepared with CPS from GBS types Ia, Ib, II, III, and V revealed that these preparations are safe and highly immunogenic in healthy adults (*7*). Although these vaccines are likely to provide coverage against the majority of GBS serotypes that currently cause disease in the United States, they do not offer protection against pathogenic serotypes that are more prevalent in other parts of the world (e.g., serotypes VI and VIII, which predominate among GBS isolates from Japanese women) (*8*). Hence, a universal protein-based vaccine against GBS is highly desirable. To date, a few potential protective antigens have been described. These include the tandem repeat–containing α and β antigens of the C protein complex (*9*) and Rib (*10*); surface immunogenic protein, Sip (*11*); and C5a-ase, a serine protease that inactivates complement factor C5a (*12*). However, of these proteins, only Sip and C5a-ase are conserved at the gene level in the majority of GBS isolates (*11*, *13*), and no systematic analysis on the extent of cross-protection is available.

To identify possible antigens suitable for use in a universal GBS vaccine, we compared the genome sequences of eight GBS strains belonging to serotypes Ia (515 and A909), Ib

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Table 1. Protection conferred by four antigens against six GBS strains assessed by active maternal immunization/neonatal pup challenge model. Female mice received three doses (days 1, 21, 35) of either 20 μg antigen or phosphate-buffered saline (PBS) combined with Freund's adjuvant. Mice were then mated, and the resulting offspring challenged with a dose of GBS calculated to kill 80 to 90% of the pups. Survival of pups was monitored for 2 days after challenge. Fluorescence given in -fold difference between cells stained with immune sera versus pre-immune sera. Protection values calculated as [(% dead in control – % dead in vaccine)/% dead in control] × 100. ND, not determined.

GBS strain	Type	Fluorescence immune/preimmune	Protein alive/treated	PBS alive/treated	Protection (%)	Statistical significance (*P* value)
			Antigen GBS 80			
515	Ia	0*	1/30	4/38	0	>0.05
7357 B	Ib	2.2	11/28	13/32	0	>0.05
DK21	II	0*	4/30	4/19	0	>0.05
COH1	III	7.5	26/29	3/29	88.5	<0.0001
2603 V/R	V	1.5	13/40	8/30	7.5	>0.05
CJB111	V	9.0	23/35	11/49	56.0	0.0001
			Antigen GBS 67			
515	Ia	10.1	19/30	5/29	55.8	0.0005
7357 B	Ib	7.8	10/20	5/34	41.2	0.01
DK21	II	8.1	27/40	9/40	58.3	0.0001
COH1	III	0*	7/30	5/30	7.6	>0.05
2603 V/R	V	2.6	5/29	7/40	2.9	>0.05
CJB111	V	11.8	29/37	1/39	77.9	<0.0001
			Antigen GBS 104			
515	Ia	0*	ND	ND		
7357 B	Ib	0	6/39	13/32	0	>0.05
DK21	II	0*	5/38	7/40	0	>0.05
COH1	III	5.6	22/40	7/33	43.0	0.0041
2603 V/R	V	0	9/30	8/30	4.1	>0.05
CJB111	V	5.8	32/48	7/26	54.3	0.0014
			Antigen GBS 322			
515	Ia	5.6	23/25	9/21	86.0	0.0004
7357 B	Ib	2.5	22/46	13/32	11.6	>0.05
DK21	II	8.4	28/40	6/24	60.0	0.0007
COH1	III	3.2	2/30	3/29	0	>0.05
2603 V/R	V	7.2	36/42	12/32	77.3	<0.0001
CJB111	V	1.4	ND	ND	ND	ND

*Gene missing in this strain.

(H36B), II (18RS21), III (COH1 and NEM316), and V (2603 and CJB111), which represent the most important disease-causing serotypes (*14*). This analysis identified a "core" genome of 1811 genes (~80% of each genome) shared by all strains and a "variable" genome of 765 genes that were not present in all strains. Computer algorithms were then used to select, within the two subgenomes, the genes encoding putative surface-associated and secreted proteins. Among the predicted surface-exposed proteins, 396 were core genes and 193 were variable genes. Of these 589 proteins, 312 were successfully expressed in *Escherichia coli* either as soluble His-tagged fusions or soluble glutathione *S*-transferase fusions.

Each purified soluble protein was next used to immunize groups of adult female mice. At the end of the immunization schedule, these were mated, and the resulting offspring (<48 hours of age) were challenged with a dose of GBS calculated to kill 80 to 90% of the pups (*14*). For conserved antigens, a virulent serotype III strain (COH1) was used for challenge; antigens that were absent from the COH1 strain were tested with one of the sequenced strains known to carry the corresponding gene. This systematic screening identified four antigens capable of significantly increasing the survival rate among challenged infant mice. One of these antigens—GBS322 (SAG0032), which encoded the previously described Sip protein (*11*)—was part of the core genome. The other three antigens—GBS67 (SAG1408), GBS80 (SAG0645), and GBS104 (SAG0649)—were present in the variable portion of the subgenome. The proportion of mice protected against challenge with strains carrying one of these proteins varied from 43% in the case of GBS104 to 80% in the case of GBS80.

Table 2. Protection against 12 GBS strains by a four-antigen combination. Experiments were performed as in Table 1 except that mice were vaccinated with a mixture of 15 μg of each protein (a total of 60 μg). Protection *P* values were less than 0.0001.

GBS strain	Serotype	Vaccine (alive/treated)	PBS (alive/treated)	Protection
515	Ia	39/40	6/40	97.0%
DK1	Ia	50/50	8/38	82.5%
7357B	Ib	49/60	5/46	79.4%
DK21	II	25/34	17/48	59.3%
5401	II	35/40	3/37	86.4%
3050	II	48/48	1/30	100%
COH1	III	36/36	7/40	100%
M781	III	30/40	4/39	72.0%
2603V/R	V	27/33	10/35	75.0%
CJB111	V	25/28	4/46	88.2%
JM9130013	VIII	37/39	5/40	94.2%
SMU071	VIII	44/50	18/50	81.2%
Total		445/498	88/498	87.0%

The four proteins were purified to homogeneity (*14*) and then tested in mice in the active maternal immunization–neonatal pup challenge model described above with the use of six GBS strains. Each antigen elicited protection against more than one strain but not against all strains (Table 1). As expected, whenever the corresponding gene was absent from the challenge strain, the antigen was not protective. However, in a few cases, protection was not conferred even though the challenge strain carried the antigen-coding gene. To test whether this is due to variability in antigen expression and/or surface exposure, we assessed antigen expression on the surface of each challenge

strain by fluorescence-activated cell sorting analysis using mouse sera specific for each of the four protective antigens. The levels of surface expression, as measured by antibody binding to viable bacteria, were variable and correlated with the protective activity of the antigen (Table 1). From the data accumulated up to this point, we estimated that an antigen was protective if antigen-specific antibody binding resulted in a >fivefold increase in fluorescence intensity over that in pre-immune controls. We then tested a combination of all four antigens in the same mouse model with the use of a panel of 12 challenge strains that represented the major pathogenic GBS serotypes and that belong to eight Multi Locus Sequence Types (MLST) (*15*). The combination of the four antigens was highly protective against all 12 strains (Table 2), with protection ranging from 59% to 100%—comparable to that conferred to mice vaccinated with CPS-tetanus toxoid glycoconjugates and challenged with homologous strains (*16*).

Lastly, we assayed the in vitro opsonophagocytic activity (*17*) of sera from mice immunized with the single antigens and with the four-antigen combination. Sera were incubated with the highly encapsulated GBS type V strain CJB111, which expresses all four antigens, and bacterial killing was measured in the presence of both polymorphonuclear leukocytes (PMNs) and rabbit complement. All sera promoted opsonophagocytosis and killing of GBS by PMNs (Fig. 1), and killing was both PMN- and complement-dependent (*14*). However, the bacteria were most efficiently killed when opsonized with sera from mice vaccinated with the combination of four protein antigens, suggesting that the four proteins work additively as potent immunogens. Taken together, the protection in mice and the opsonophagocytic activity of the mouse sera suggest that a vaccine based on these four antigens may confer effective protection in humans also.

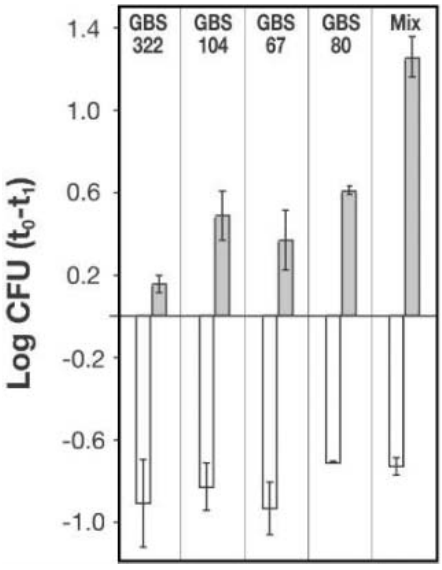

Fig. 1. Opsonophagocytic activity of sera specific for vaccine antigens. Live GBS bacteria of strain CJB111 were incubated for 1 hour with human PMNs in the presence of baby rabbit complement and specific antisera. The $\log_{10}$ of the difference between bacterial colony forming units at time = 0 and time = 1 hour are shown. Values for preimmune sera are negative because of bacterial growth during the assay. The antigens used are recorded above each bar. Shaded bars represent specific immune sera; open bars, the corresponding preimmune sera from the same animals. Error bars indicate standard deviation.

At least two major conclusions can be drawn from this work. First, multistrain genome analysis and screening constitute an effective new approach to identifying vaccine candidates that can provide broad protective activity when used in combination. Of the four antigens identified, none could be classified as universal because, in a fraction of GBS strains, either their coding gene was absent or their surface accessibility was negligible. Therefore, a genome screen of a single strain (*18*) would not have led to the identification of all four antigens but would have identified only those that, by coincidence, were sufficiently expressed in the strain used for challenge in the mouse model.

Despite the absence of universal antigens, it is clear that appropriate combinations of protective antigens—each effective against overlapping populations of isolates—can confer unexpectedly broad serotype-independent protection. In fact, the four-antigen vaccine used in this work protected mice against 12 virulent strains belonging to all nine major GBS serotypes. To estimate the strain coverage of the vaccine, we analyzed the surface expression of the four antigens on a total of 37 GBS isolates. We found that at least one of the antigens was highly accessible to antibodies (>fivefold shift in fluorescence) in 32 out of the 37 strains tested, which corresponds to 87% of circulating strains assuming that these strains sufficiently reflect the variability in the population.

A second conclusion from this work is that the extent of surface accessibility of antigens may vary from strain to strain, even if the antigens' coding genes are conserved (Table 1). Such variability may be due to differences in gene expression, antigen masking by other cellular components (e.g., CPS), protein degradation, or other factors. For instance, we found that the surface accessibility of the protein Sip was dependent on the presence of the polysaccharide capsule (table S2). In line with this, the protective antigens we identified were effective only against those strains in which the antigens were sufficiently exposed on the bacterial surface. From a practical point of view, variability in surface antigen expression highlights the importance of upfront rational selection of strains to be used in protection models. The strains should be selected not only because they carry the gene for the antigen under examination, but also in light of the amount of expression and accessibility of the antigen itself. Between 30 and 40% of the genes of all bacteria sequenced so far belong to hypothetical or unknown families. Because our approach selects antigens independent of their function, it was likely that some protective antigens would have no assigned function. This is the case for all four protective antigens described herein. GBS322 contains a LysM domain, which is found in a variety of enzymes involved in bacterial cell-wall degradation and may have a general peptidoglycan-binding function. GBS67, GBS80, and GBS104 all contain LPXTG (Leu-Pro-X-Thr-Gly, where X is any amino acid) motifs associated with covalent linkage to the cell wall (*19*). Indeed, we have recently found that all three proteins are components of pilus-like structures never described before in GBS (*20*).

In GBS and probably other bacterial pathogens that adopt the strategy of gene variability to escape the immune system, universal protective protein antigens are unlikely to exist. However, some protein antigens are conserved in sufficiently large subpopulations of GBS that in combination they can be broadly protective. The successful use of multistrain genome analysis and screening described here for GBS provides the basis for the potential development of universal protein-based vaccines against other important and highly variable pathogens such as Group A *Streptococcus* and *S. pneumoniae*.

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Supplementary Online Material
www.sciencemag.org/cgi/content/full/309/5731/148/DC1
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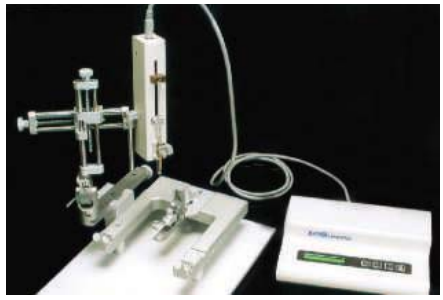

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An Overview of Recent Advances Although many scientists point to the Human Genome Project as the leading milestone of recent years, many other advances—such as array technology and RNA interference—also push ahead basic and applied research. The experts interviewed here explore recent developments in knowledge and techniques. BY MIKE MAY AND GARY HEEBNER

In this issue:

- Microarrays
- Microfluidics
- RNA interference
- Primary cells
- Drug screening
- Physiological simulation

Life science moves ahead rapidly, and has for the past couple decades. In fact, life science advances so fast that telling a milestone from just another incremental achievement can take some thinking. Fortunately, Donald Kennedy, editor-in-chief of ***Science***, provides two guidelines. He says, "One feature of a milestone is the development of really new technology that lets scientists do things that they never could before that advance." Such advances include fluorescent microscopy and high throughput genomic sequencing. "The second feature worth pointing out about milestones," says Kennedy, "is a discovery that brings into play a whole assembly of new technology to do something that we would very much like to do." For example, it would surely be a milestone in life science to combine genomics, drug discovery, and other approaches to fight human disease or aging (for more on aging see the accompanying Updates on Aging).

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In the past five years, though, selecting the key milestone in life science seemed easy enough for the experts interviewed here. They all pointed out the sequencing of the human genome. Many also added sequencing model organisms as equally important. Other milestones emerged, as well. For example, Kennedy indicates the value of small interfering RNAs. He says, "They provide the capacity to knock down rather than knock out gene function, and that lets us explore all the ways that RNAs may influence control over transcription." The experts interviewed here also mentioned the value of automation, microarrays, microfluidics, and other technological advances that drive life science.

The advances, however, go beyond molecular-based science. Improvements also push ahead behavioral sciences and ecology. For example, a combination of electronic tags and MORE ›››

» milestones in: Life Science

satellites recently helped a team of scientists from the Hopkins Marine Station track Atlantic bluefin tuna. Other behavioral scientists now use robots to tease out details behind displays of aggression and communication.

As the experts interviewed here reveal, life science quickly moves to levels never imagined. Read on to hear about some exciting recent advances and possible advances that lie ahead.

Ever-Improving Arrays

Some of the major milestones in recent years for the life sciences come from processing more samples of smaller size, which can be done, for example, with microarrays. Steve Lombardi, senior vice president, product marketing and development at **Affymetrix**, ranks microarrays among the top five milestones of the past five years. He says, "The world of life science used to think of a genome as a list of genes. Microarrays enable whole genome science by showing us that there is a molecular biology of the genome." The devices can be made in a laboratory or purchased ready to use from companies such as Affymetrix. Companies specializing in the fabrication of microarrays include **GE Healthcare**, **Hitachi Genetic Systems/MiraiBio**, and **Thermo Electron**.

These devices also provide new ways to study disease. Lombardi explains that high density arrays let scientists look at disease-related sequence variation, see what genes get turned on with a disease, and understand the transcription factors that bind to DNA and turn on the genes in the first place. He adds, "The amount of information we can now put on one Affymetrix chip—over 6 million probes—means that you can do all of this on one chip on one platform." Moreover, this approach is unbiased and hypothesis-free. That is, a scientist lets the experiments reveal how the genome works.

Microarrays can also be aimed specifically at signal transduction research, especially with products from **EMD Biosciences** and **SuperArray Bioscience**. Li Shen, president of SuperArray, says, "The microarray is a revolutionary concept. It changes how we can get information from a cell."

For example, SuperArray's GEArrays can be used to validate and confirm a hypothesis, especially one related to a disease. Shen says, "We have quite good coverage for different applications. We have about 50 arrays for pathways of diseases, including cancer, immunology, neuroscience, toxicology, stem cells, and developmental biology."

In addition to making microarrays for many applications, SuperArray also aims to make this technology simpler to use. She says, "Another technical focus for us is making microarrays reliable and easy to use. We want microarrays used in every lab as a routine tool, like the polymerase chain reaction [PCR] is used today."

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Fine Tuning the Fluid Flow

Microfluidics handles very small volumes of fluids, including nano- and picoliter volumes, for DNA analysis, the separation of human blood cells, and other applications. This field includes lab-on-a-chip technology, which comes from several companies, including **Agilent Technologies**, **Caliper Life Sciences**, **Cepheid**, and **Gyros**.

According to Kevin Hrusovsky, president and chief executive officer of Caliper, "Lab-on-a-chip technology might not have been a milestone in the past five years, but it will be in the next five." He adds that as many as 70 percent of the top-tier big pharmaceutical companies use this technology.

For example, Caliper's LabChip 3000 drug discovery system miniaturizes, integrates, and automates enzymatic and cell based assays. Hrusovsky says that this instrument is being used by Johnson & Johnson, Merck, Pfizer, sanofi-aventis, and other big pharmaceutical companies that need higher quality screening and profiling data. He says, "The LabChip 3000 is very robust, much less expensive, and we have not had a chip-quality return in over a year."

Advances in drug discovery will also come from new approaches to protein identification and quantification. For example, the disposable Prespotted AnchorChip—a matrix assisted laser desorption/ionization-time of flight (MALDI-TOF) target—from **Bruker Daltonics** can help researchers find new lead compounds or biomarkers. According to Detlev Suckau, head of application development for MALDI-TOF instruments and proteomics at Bruker, "This chip provides a 10-fold to 100-fold increase in sensitivity." It consists of a plastic substrate—developed by **Eppendorf** and Bruker Daltonics—with prespotted MALDI matrix and calibrant spots. Suckau says, "Eppendorf has the technical expertise to produce injection molded plastic chips of the required quality for this product. So it was an excellent choice to work with them."

A Gene Take Down

When scientists first learned about genes, they surely thought of finding ways to block them. A variety of systems—including genetically engineered lines of mice, altered tissue culture cells, and molecules like short interfering (si) RNA—suppress or knock down genes. MORE ›››

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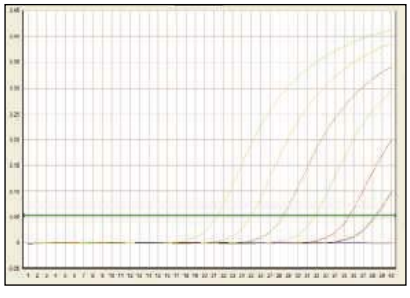

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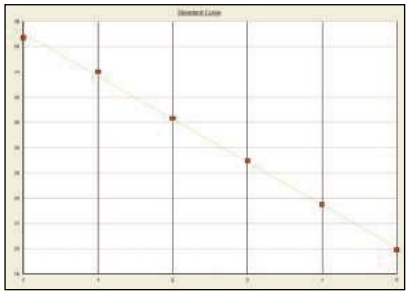

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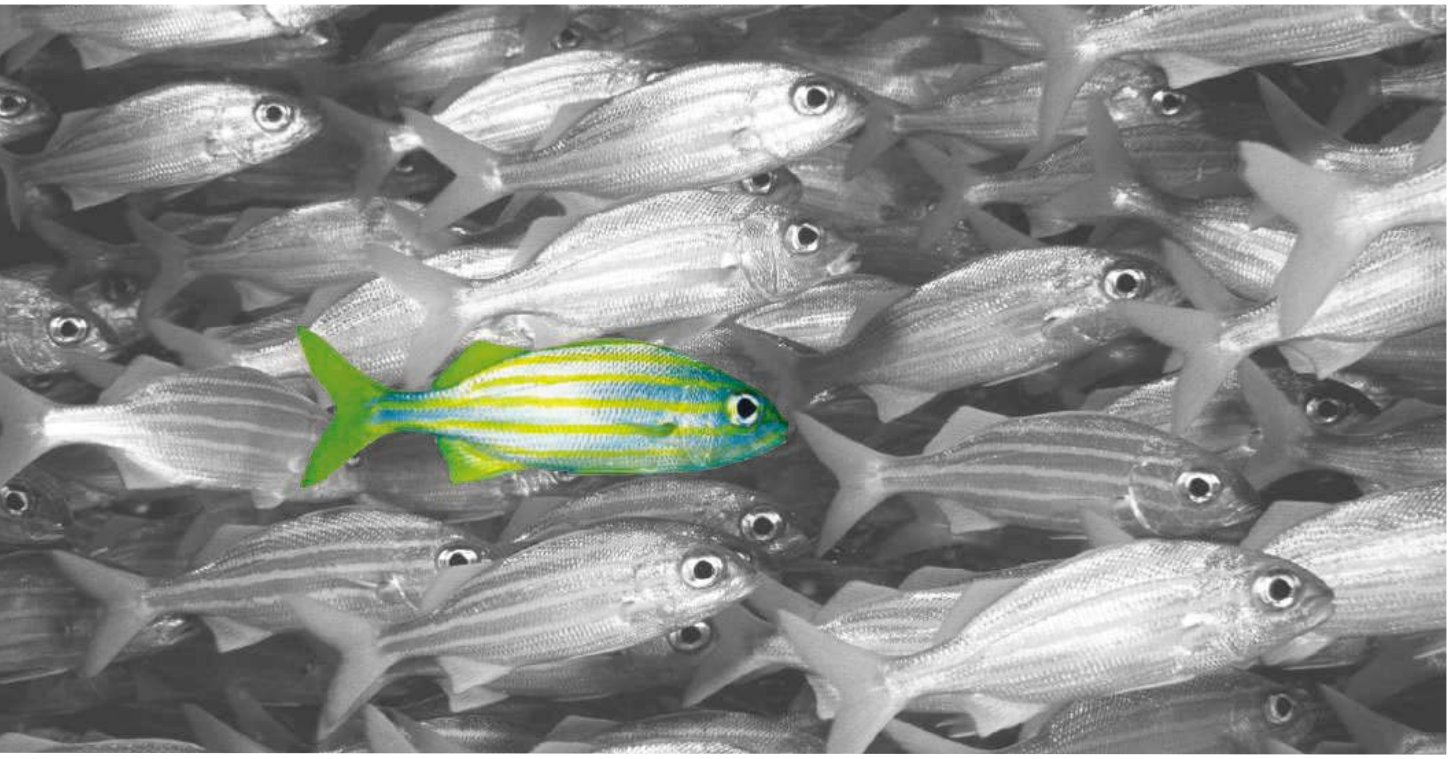

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» milestones in: Life Science

Among the experts asked here, and probably many life scientists, RNA interference (RNAi) ranks as an important milestone.

To cause RNAi, a scientist puts double stranded RNA into a cell. The double stranded RNA matches up with complementary mRNA and destroys it, which stops it from making proteins. So far, scientists know that RNAi takes place in cells from many organisms, including mammals, and it apparently participates in antiviral defense and the regulation of gene expression. A number of companies—including **Ambion**, **Dharmacon**, and **New England Biolabs**—offer RNAi kits.

David Brown, research and development senior scientist and head of the siRNA project group at Ambion, believes that RNAi filled a research void. He says, "Genome projects verified the blueprint of life—all the genes—but the key is to understand how they interact and function. RNAi provides the opportunity to link gene identity with gene function. You can eliminate a gene's expression and see what happens."

This technique can be used in basic and applied research. For example, RNAi can be used to fight disease. A researcher can knock down individual genes and see how it affects the phenotype of different cells. For instance, says Brown, "Cancer researchers can look for gene-specific siRNAs that affect proliferation or apoptosis or angiogenesis or telomerase activity." In addition, these techniques can be applied to high throughput assays. Brown says, "We now have delivery techniques for cell lines and primary cells that provide highly reproducible knock down in every well of a 96- or 384-well plate."

In many experiments, scientists would prefer to focus on primary cells. According to Pat Dillon, president and chief executive officer at **ArtisOptimus**, "People have always known the importance of primary cells." Still, researchers often used immortalized cells instead, mostly out of convenience. "But immortalized cells are not normal," says Dillon. "Some are a lot farther from normal physiology than you would like for studies." For example, immortalized cell lines can grow differently than cancer cells in the body.

To give researchers access to primary cells, ArtisOptimus grows primary mouse embryo fibroblasts (MEFs) from normal and knockout mouse models that retain their initial growth and genetic properties. The company offers knockout MEFs for several areas related to cancer research: cell cycle, apoptosis, and signaling studies.

Moreover, providing primary cells with known gene knockouts will open new approaches to studying disease. For instance, Dillon says, "Certain MEFs have been used in studies of growth, which can be applied to cancer biology." In addition, primary cells can be used for drug development. As Dillon says, "We have generated some knockout MEFs that include genes involved in drug transport. With these, a researcher can look at a drug's metabolic effects."

Updates on Aging

SAGE KE—the Science of Aging Knowledge Environment—offers one-stop shopping for investigators interested in the science of aging. This site features scientist-written reviews, perspectives, as well as case studies on neurodegenerative diseases. In addition, it delivers new stories on the latest discoveries and orientation articles on hot topics in the field. This website also provides meeting information and a variety of other useful sections.

» http://sageke.sciencemag.org

Screening and Simulation

In the world of biotechnology and pharmaceutical companies, researchers remain on full-time alert for improved throughput in drug screening. Although that process keeps picking up speed, Jeff Mooney, commercial technology director in the Life Sciences Division at **Corning**, says, "You can now run through the targets quickly, but you still have a bottleneck of both false positive and negative 'hits,' and the inability to screen intractable or orphan targets."

To enhance the accuracy of screening, Corning developed its Epic platform. Mooney says, "We surveyed bottlenecks from identifying viable targets to validating leads, and then designed the Epic system to solve some of these problems." That produced a label-free, high throughput system that detects biomolecular interactions. For instance, this platform identifies interactions between proteins, proteins and small molecules, and chemicals—all on a 384-well plate.

After releasing this product, Mooney started receiving positive feedback from customers. He says, "We have people telling us that an assay which took six months to develop for conventional screening systems can be done in just a week with our system." In addition, Mooney says, "We are currently working with customers on assays for intractable targets as well as on assays to validate a reduction in both false positive and negative hits. Better lead generation will reduce costs and hopefully enable better drugs."

Some of today's experimental research can take place in silico. That is, interactions, biologic processes, and system behaviors can be modeled on a computer. Mikhail Gishizky, chief scientific officer at **Entelos**, says, "Use of computer biosimulation is in its infancy for life sciences." He adds, "Computer simulation will play a greater role in understanding qualitative and quantitative differences in biologic MORE ›››

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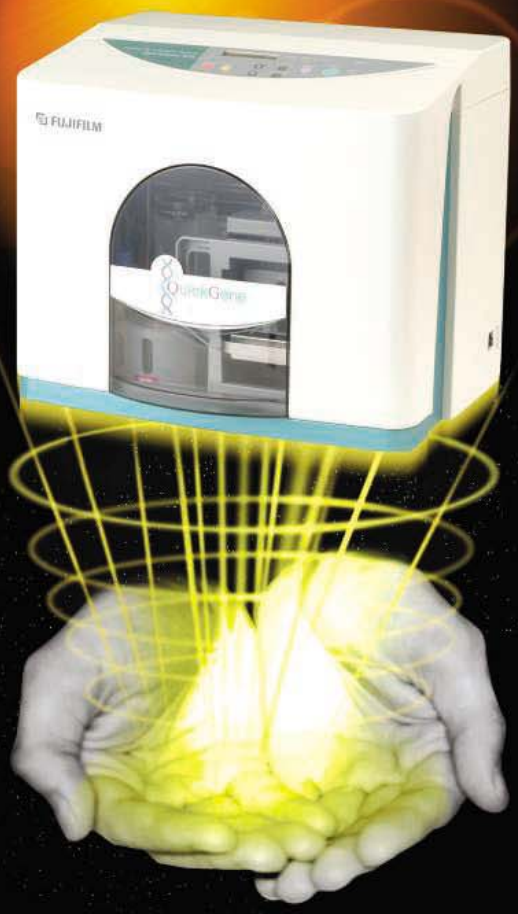

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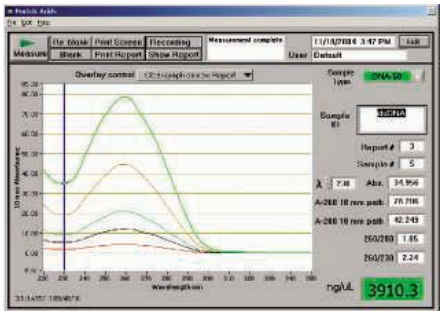

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processes between, for instance, mouse and man, and help scientists make better decisions." At Entelos, scientists build quantitative models that predict human biological behavior. Specifically, this company's PhysioLab technology enables the development of large-scale, whole-system models of dynamic biological behavior. In fact, this modeling system is being used in many projects with pharmaceutical companies, including finding druggable targets and designing clinical trials.

The greatest advances of the recent past required combinations of technologies. Even more integration might lie ahead, especially in fields like systems biology. Kennedy says, "It's very hard to predict what new technologies will be possible even in the near future." As an example, though, he says, "Applications of lab automation are going to outstrip our capacity to even envision what they might be." Although he worked in neurobiology for many years, he adds, "When I walk into a neurophysiology lab today, I see people using computers to manage experiments in ways that I would not have dreamed possible." Perhaps a scientist's dreams are the very things that lead to milestones.

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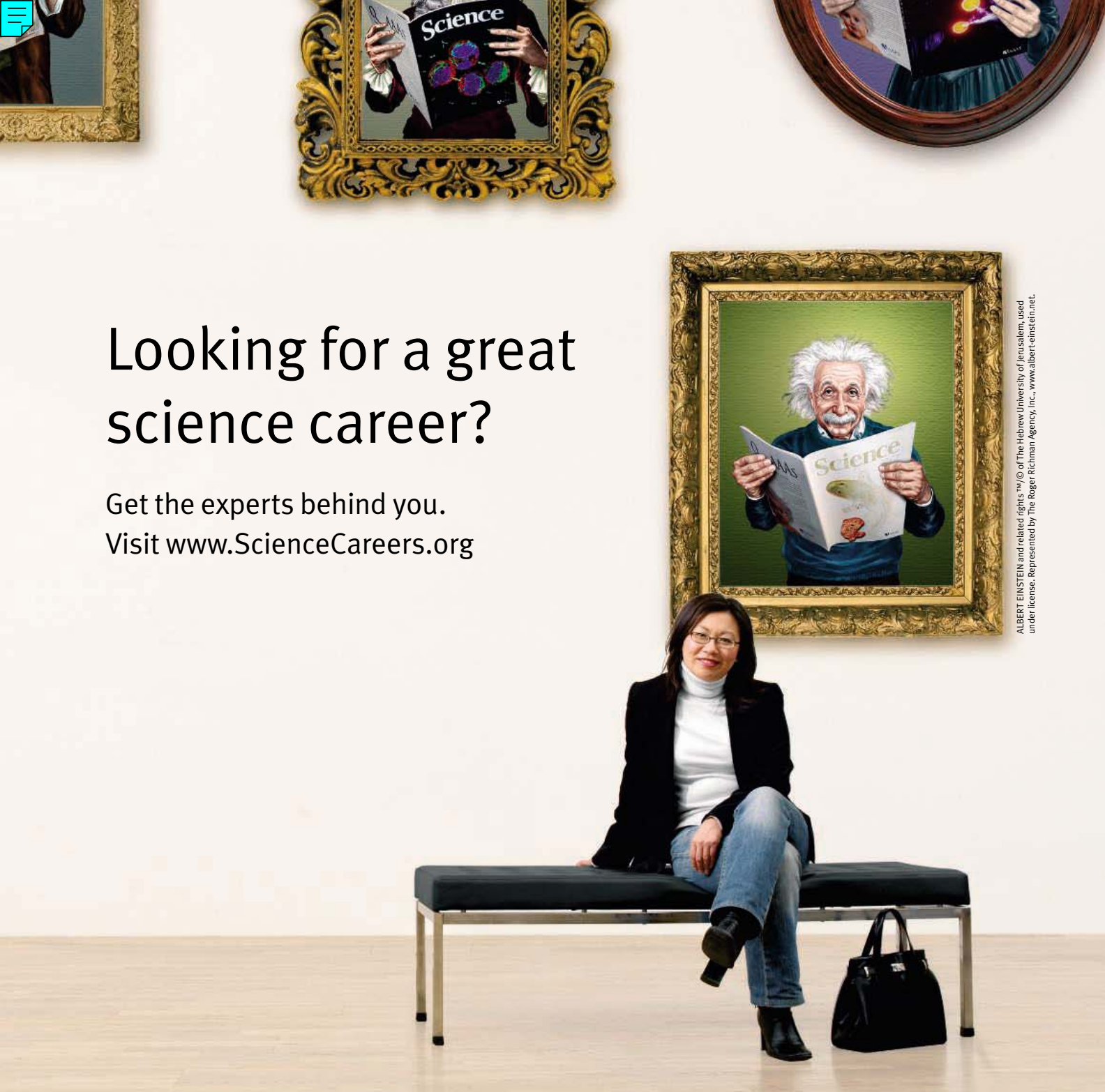
New England Biolabs, Inc., kits and reagents for RNAi research, http://www.neb.com

SAGE KE – the Science of Aging Knowledge Environment, website for aging research, http://www.sageke.sciencemag.org

SuperArray Bioscience Corporation, microarrays for signal transduction research, http://www.superarray.com

Thermo Electron Corporation, products for microarray fabrication, http://www.thermo.com



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POSTER INSIDE

MILESTONES OF SCIENCE

In celebration of the 125th anniversary of the journal, *Science*, AAAS is publishing this illustrated timeline of discovery to inspire interest in learning more about major scientific innovations throughout history. The poster highlights 129 top milestones, emerging over the course of 2,600 years, which advanced research and helped evolve the scientific community into what it is today. It's designed for curious kids and adults to discover science together in a classroom, living room, or lab. We thank our sponsor Corning for making possible the distribution of this poster in *Science*.

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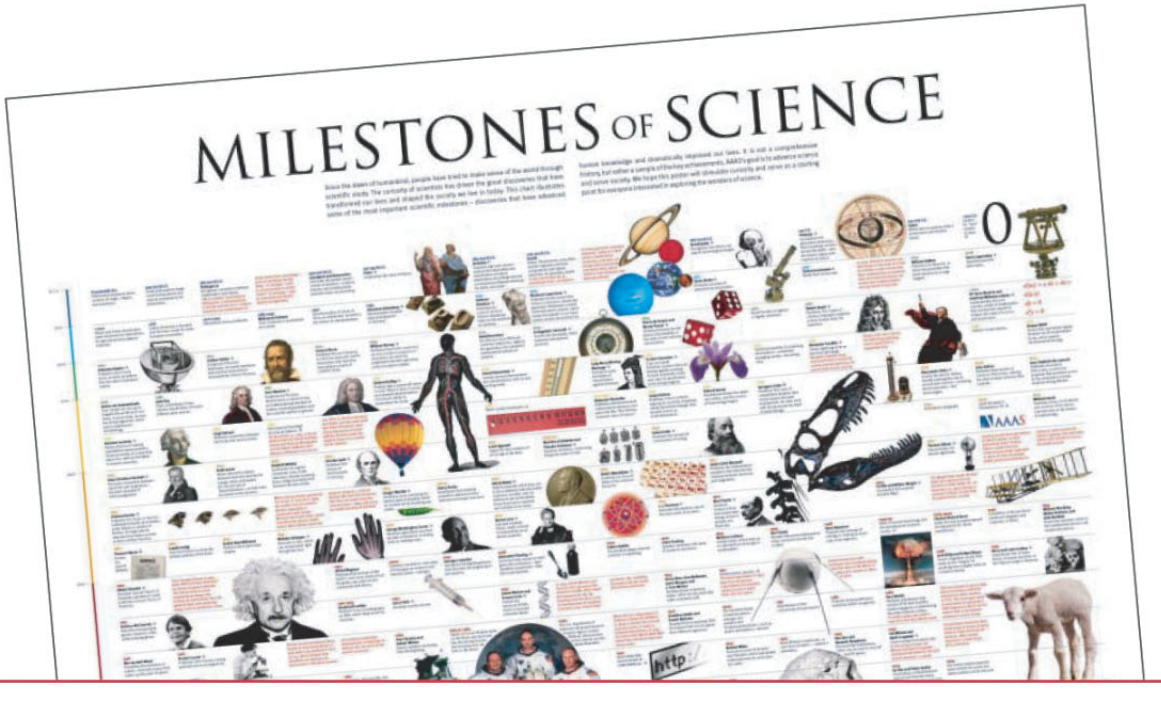

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Science AAAS

MILESTONE

Since the dawn of humankind, people have tried to make sense of the world throu
scientific study. The curiosity of scientists has driven the great discoveries that ha
transformed our lives and shaped the society we live in today. This chart illustrat
some of the most important scientific milestones – discoveries that have advanc

B.C.E.

Prescientific Era
Phenomena explained within contexts of magic, religion, and experience.

599–500 B.C.E.
Greek philosophers begin constructing natural and rational explanations for phenomena.

582–500 B.C.E.
Pythagoras
Recognizes connection between mathematics and nature.
"All is number," according to Pythagoras, a mathematician in the 6th century B.C.E. Today's scientists use mathematics to study topics from cosmology—such as modeling the Big Bang—to ecology—such as using fractals to model nature—to zoology—such as unraveling the family history of organisms.

499–300 B.C.E.
Leucippus and Democritus
Articulate the earliest known version of atomism—a theory that tiny and indestructible particles make up everything.

427–347 B.C.E.
Plato →
Emphasizes the value of theory.

384–322 B.C.E.
Aristotle ←
Supports logic and common sense in the observation and classification of natural phenomena. His writings become a cornerstone of Western thinking for nearly 2000 years.

299–200 B.C.E.
Euclid
Writes *The Elements*, one earliest logically built te using axioms and logical deduction for geometric
In the 3rd century B.C.E. alchemists tried—and fa

1500

~1000
Mayan and Hindu skywatchers make astronomical observations for agricultural and religious purposes.

1166
Oxford University is founded and becomes model for other European universities.

1200–1299
Mechanical clocks proliferate.

1285–1349
William of Ockham
Touts simplicity in explanation as a virtue.

1370
Synchronization of clocks in Paris, an emblematic moment in the history of standardization.

1454
Johannes Gutenberg →
Demonstrates movable type printing press in Germany.

1543
Andreas Vesalius →
Publishes *De Corporis Humani Fabrica*, displacing Galen as the authority on anatomy.

1543
Nicolaus Copernicus →
Publishes *De Revolution Orbium Coelestium*, disp geocentric (Ptolemaic) s with heliocentric system means the planets in ou system revolve around th

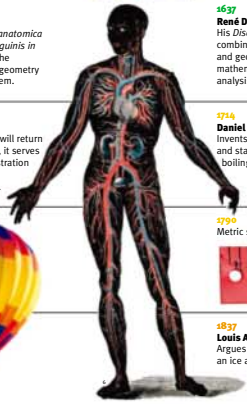
1600

1609
Johannes Kepler →
Publishes *Astronomia Nova*, in which he defines first two laws of planetary motion.

1610
Galileo Galilei →
Using one of the first telescopes, he makes extensive astronomical observations and publishes *Siderius Nuncius*.

1620
Francis Bacon
Publishes *Novum Organum*, in which he espouses careful observation and inductive reasoning as a basis of scientific study.

1629
William Harvey →
Publishes *Exercitatio anatomica de motu cordis et sanguinis in animalibus*, by which he establishes the basic geometry of the circulatory system.



1637
René Descartes
His *Discours de la Methode* combines skepticism, algebra, and geometry into a powerful mathematical method of analysis.

1643
Evangelista Torricelli →
Invents the barometer, w measures atmospheric pressure.

1700

1676
Anton van Leeuwenhoek
Uses simple microscope to magnify the world up to 200 times. In pond water, he sees tiny living organisms, which opens a pathway to microbiology.

1686
John Ray
Starts publishing a three-volume classification of 18,600 different plant species.

1687
Isaac Newton ←
Publishes his *Principia Mathematica*, in which he lays out mathematically the laws of motion, universal gravitation, and the scientific method in general.

1705
Edmund Halley ←
Predicts that a comet will return in 1758. When it does, it serves as a powerful demonstration of science's ability to understand the world.

1714
Daniel Fahrenheit →
Invents mercury thermometer and standardizes it with ice and boiling water.

1769
Antoine Lavoisier →
Raises practice of making quantitative measurement in chemical studies to a level that earns him credit as the father of modern chemistry.

1780
Luigi Galvani
Observes connection between electricity and muscle action.

1783
First manned free flight of a hot air balloon. →
On November 21, 1783, a hot air balloon built by French brothers Joseph and Etienne Montgolfier carried Pilatre DeRozier and Marquis D'Arlandes into the air over Paris in the first manned, free flight. Just two years later, two other Frenchmen, Jean-Pierre Blanchard and John Jeffries, floated across the English Channel in another hot air balloon.

1790
Metric system developed. ↓

1800

1820
Hans Christian Oersted →
Discovers connection between electricity and magnetism—the basic principle of electromagnetism.

1824
Sadi Carnot
Writes about the relation between heat and mechanical energy, which anticipated the Second Law of Thermodynamics—or that order decreases in the universe.

1828
Friedrich Wöhler
Synthesizes an organic compound, urea, in the laboratory, thereby breaking down categorical distinction between life and nonlife.

1830
Charles Lyell →
Publishes first volume of *The Principles of Geology*.

1837
Louis Agassiz
Argues for the existence of an ice age in the past.

1838–39
Matthias Schleiden and Theodor Schwann →
Develop cell theory—tha things are made of cells.

1859
Charles Darwin →
Publishes *the Origin of Species*, outlining his theory of evolution, partly based on studies of finches. Through natural selection their beaks evolved to best suit their function.

When Charles Darwin published The Origin of Species *in 1859, the modern era of evolution started, especially in conjunction with Gregor Mendel's work with peas, which revealed how species might evolve. More details about the behavior of genes—such as Barbara McClintock's discovery that they can jump along the chromosomes—keep improving our understanding of heredity.*

1865
Gregor Mendel ↓
Publishes work containing his famous laws of heredity, which he worked out by breeding pea plants.

1860s
Henry Sorby
Emphasizes how material properties depend on their internal microscopic structure.

1866
Alfred Nobel →
Patents dynamite, which turns out to be a relatively safe and useful explosive. He later uses his fortune to set up the Nobel Prizes that honor scientists and scholars in six areas.

1880
Thomas Edison ↓
Founds the journal *Science*.

1889
Camillo Golgi
Uses techniques to reveal the cellular anatomy of the brain.

1893
Daniel Hale Williams
Performs first open heart surgery.

1895
Wilhelm Röntgen →
Discovers X-rays, which can reveal bones right through the skin.

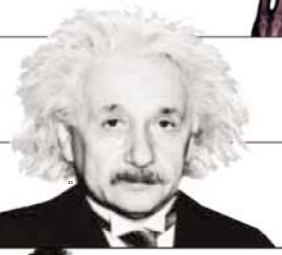
1896
George Washington Carver →
Advances agriculture over four decades of research, including work on rotating crops.

1897
Marie Curie →
She and husband, Pierre, clarify concept of radioactivity as an atomic property.

1900

1905
Albert Einstein →
Develops Special Theory of Relativity, and later (in 1916) publishes *General Theory of Relativity*.

Time changed forever in 1905, when Albert Einstein described his theory of special relativity. This theory leads to some constraints on nature, like nothing can travel faster than the speed of light, and surprising things, such as variations in time.



1912
Alfred Wegener
Proposes that sections of the Earth's crust move slowly across the globe, and calls this the continental drift theory.

1920s
Quantum mechanics—the study of what goes on inside of and between atoms—emerges.

1927
Georges Lemaître
Introduces the Big Bang theory that describes the beginning of the universe.

1928
Alexander Fleming →
Discovers that a strain of mold gives off a substance—later identified as penicillin—that kills some bacteria.

1951
Barbara McClintock →
Describes transposable genetic elements, often called jumping genes.

1951–1953
Rosalind Franklin
Performs X-ray crystallography on DNA, which helps unveil its structure.

1952
Jonas Salk →
Develops a polio vaccine.

1953
James Watson and Francis Crick →
Publish the structure of DNA, which is basically a twisted ladder.

In 1953, James Watson a Francis Crick uncovered structure of DNA, often c the blueprint for life. By knowing how the basic u of chromosomes fit toge scientists soon solved o

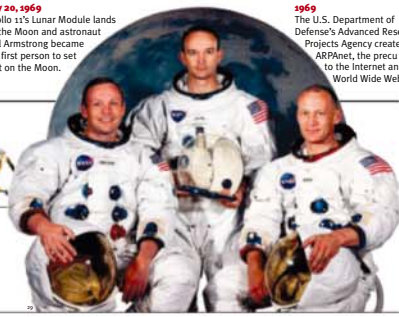
1961
Murray Gell-Mann
Postulates the existence of quarks—basically a piece that makes up the parts of atoms.

1962
Rachel Carson →
Publishes *Silent Spring*, linking pesticide use to ecological ills.

Rachel Carson introduced many people to environmentalism in 1962 when she published Silent Spring, *which showed how chemicals can damage our environment. That work evolved into today's sustainable development, or attempts to balance the use of all of the world's resources.*

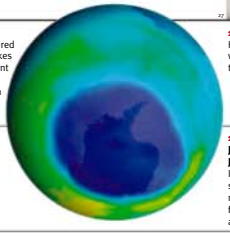
1964
Arno Penzias and Robert Wilson
Detect radiation remaining from the Big Bang.

July 20, 1969
Apollo 11's Lunar Module lands on the Moon and astronaut Neil Armstrong became the first person to set foot on the Moon.



1969
The U.S. Department of Defense's Advanced Rese Projects Agency create ARPAnet, the precu to the Internet an World Wide Wel

1985
Ozone hole discovered over Antarctica makes a dramatic statement about the effect of human activities on the environment. →



1990
Human Genome Project begins with a goal of sequencing all of the human chromosomes.

1990
Seiji Ogawa
Describes blood oxygenation level dependent contrast, or BOLD, fMRI method, which records activity in the brain.

1991
Two hikers discover Ötzi, the Iceman of the Alps, who turns out to be 5,300 years old—the oldest frozen mummy ever found.

1997
Universe dated at 13–14 billion years old from expansion rate.

1998
John Gearhart and James Thomson
Independently grow embryonic stem cells that might be used to make any other kind of cells, from bone and blood to muscles and neurons.

2001
Draft sequence of human genome published. ←
In 2001, the Human Genome Project and Celera, the latter group's study published in Science, *unveiled the order of pieces, or sequence, of the roughly 30,000 genes in human chromosomes. This work opened the way to a future of therapies designed just for you—personalized medicine.*

2000

Editorial note: A chart of milestones originally published in *Science* on January 14, 2000 (volume 287, issue 5451) made up the basis for this chronology. Freelance science writer and editor Mike May, Ph.D., developed additional listings and featured topics. This chart was prepared and produced by the Office of Publishing and Member Services. Please address any questions and comments to: memuser@aaas.org

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human knowledge and dramatically improved our lives. It is not a comprehensive history, but rather a sample of the key achievements. AAAS's goal is to advance science and serve society. We hope this poster will stimulate curiosity and serve as a starting point for everyone interested in exploring the wonders of science.

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cts
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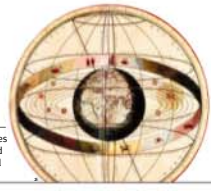
proofs.
ailed—

to make gold from everyday elements. Today, in a field called combinatorial chemistry, scientists can design new compounds and make them, which leads to new materials and pharmaceuticals.

287–212 B.C.E.
Archimedes →
Recognizes how theory can lead to technological insight.

140 C.E.
Ptolemy →
Summarizes his geocentric astronomy—that everything revolves around the Earth—and this theory reigns until Copernicus (1543).



131–200 C.E.
Galen
Writes text on anatomy that is preeminent until Vesalius (1543).

~500 C.E.
Symbol for "zero" created in India. →

ibus
lacing
ystem
, which
r solar
he sun.

1570
Tycho Brahe ←
Increases accuracy of astronomical observation.

1590
Zacharias Janssen ←
Builds first microscope.

In 1590, Zacharias Janssen put two lenses at the ends of a tube and made a crude microscope. Now, combinations of chemical dyes and advanced optics can reveal color-coded proteins moving inside living cells.

1600
William Gilbert
Publishes *De Magnete*, in which he postulates that Earth behaves as a huge magnet.

1608
Hans Lippershey →
Manufactures first telescopes.

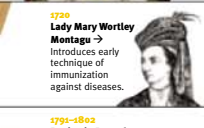
hich

1654
Pierre de Fermat and Blaise Pascal →
Develop the basis for the theory of probability, or the odds of one outcome over another.

1660
Royal Society in England is legally chartered.

1661
Robert Boyle →
Publishes *The Sceptical Chymist*, helping to establish a more modern basis for chemistry.

~1670
Sir Isaac Newton and Gottfried Wilhelm Leibniz →
Independently discover calculus, a field of mathematics that can find the volume of shapes, the slope of a curve, and much more.



1720
Lady Mary Wortley Montagu →
Introduces early technique of immunization against diseases.

1735
Carolus Linnaeus →
(Carl von Linné)
Publishes *Systema Naturae*, thereby greatly pushing forward systematic study and rules of classification of the living kingdom.

1750
Statistical benefits of combining observations—essentially averaging results—becoming clear.

1752
Benjamin Franklin →
Shows lightning to be an electrical discharge.
Benjamin Franklin long suspected that an electrical current created lightning. He proved that in June 1752 by flying a kite into a storm and watching lightning send a spark from a suspended key.

1758
Halley's Comet returns.

1759
Kaspar Wolff
Shows that specialized organs develop out of unspecialized tissue, which spawns developmental biology.

1791–1802
Benjamin Banneker
Publishes astronomical and mathematical almanacs and earns the title "first African-American man of science."

1795
James Hutton
Publishes *Theory of Earth*, arguing for a picture of gradual, steady terrestrial changes that became known as uniformitarianism.

1796
Edward Jenner
Develops technique he called vaccination, and the science of immunology begins.

1797
Georges Cuvier ↓
Publishes first work of comparative anatomy that would support the later evolutionists. His later work with fossils founds the field of paleontology.

1806
Alessandro Volta ←
Invents the electric battery, thereby opening the way to several new sciences—including electrochemistry—and technologies.

1808
John Dalton
Publishes *New System of Chemical Philosophy*, refining the idea of atoms and how they combine.

1809
Jean-Baptiste de Lamarck
Publishes *Zoological Philosophy*, in which he proposes that evolution occurs by the inheritance of traits acquired during lifetime.

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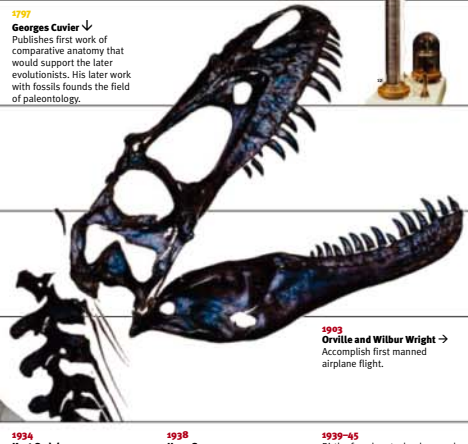
1840
James Joule →
Develops the concept of conservation of energy.



1844
First electric telegraph.

1848
AAAS founded in Philadelphia, PA. ↓

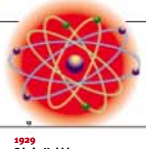
1856
William Ferrel
Publishes *Essay on the Winds and Currents of the Ocean*, a pivotal work in the history of meteorology.

1869
Dmitri Mendeleev →
Publishes his *Periodic Table of the Chemical Elements.*

1873
James Clerk Maxwell
Publishes the mathematical equations that describe the interplay between electricity and magnetism.

1879
Thomas Edison →
Demonstrates his electric light bulb.

Lighting turned from gas flames to electric glows, at least potentially, when Thomas Edison created the first commercially viable light bulb in 1879. He also developed power plants and switches to create an entire system for electric lighting. One year later, Edison also founded Science, *the AAAS journal.*



1897
J. J. Thomson ←
Discovers the electron, one of the basic parts of an atom.

1900
Max Planck →
Quantum science is born and reveals that energy comes in packets of a fixed size.

1903
Orville and Wilbur Wright →
Accomplish first manned airplane flight.

The Wright Flyer made the first flight of a heavier-than-air machine at Kitty Hawk, NC, on December 17, 1903. During 12 seconds of flight, Orville flew the plane about 120 feet.

1929
Edwin Hubble
Successfully argues that the universe is expanding.

1930
Linus Pauling
Updates chemistry with ideas of quantum mechanics.

1934
Wallace Crothers
Invents nylon, which ends up in clothing from stockings to windbreakers.

1934
Kurt Godel
Reveals that some mathematical ideas cannot be proved true or false.

1938
Hans Spemann
Proposes the concept of cloning, or making an exact copy of an organism.

1939–45
Birth of nuclear technology and the development of atomic bombs. ↓

Early 1940s
Charles Richard Drew
Leads the way to producing and storing plasma for blood transfusions.

1946
Completion of the electronic numerical integrator and computer, or ENIAC.

1947
William Shockley, Walter Brattain, and John Bardeen
Invent the transistor, which is the fundamental device in all modern electronics.

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problems, too, including how genes file the recipe for proteins and more.

1953
Harry Hess, Dan McKenzie, Jason Morgan, and J. Tuzo Wilson
Develop theory of tectonic plates, which are the parts that make continents drift.

1957
Soviets launch *Sputnik*. →
The space age began in 1957, when the Soviets put a basketball-size satellite called Sputnik in orbit. Then, the U.S. space program took over with successes that included putting the first man on the Moon in 1969 and a lander on Mars in 1976.

1958
Jack Kilby and Robert Noyce
Invent integrated circuits that make up the "engine" of anything from a digital watch to a supercomputer.

1959
Mary and Louis Leakey →
Unearth the first of many fossils of human ancestors in the Olduvai Gorge in Tanzania.

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When the U.S. Department of Defense created the ARPAnet in 1969, it started the concept of going online, at least in isolated laboratories. In 1992, the birth of the World Wide Web aimed the Internet at labs and homes alike.

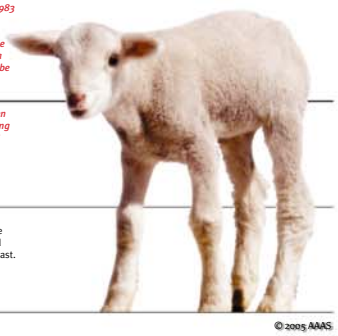
1970
Hamilton Smith and Daniel Nathans
Develop first recombinant DNA techniques that can mix genes from different organisms.

1970s
The Standard Model of particle physics emerges and explains how fundamental particles—such as quarks and leptons—interact.

1971
Manufacture of first commercial microprocessor.

1981
Acquired immunodeficiency syndrome (AIDS) recognized.

1983
Kary Mullis
Develops polymerase chain reaction (PCR) that simplifies sequencing genes, or determining the order of pieces in them.
To study a gene, you need a copy of it, and Kary Mullis made that much easier to get in 1983 with a technique called the polymerase chain reaction, or PCR. This technique made it much easier to generate a DNA fingerprint, which can be used in labs or as forensic evidence in courts of law.



1992
World Wide Web demonstrated at CERN.WWW.net. →

1993
Andrew Wiles
Announces proof of Fermat's Last Theorem, which had eluded mathematicians for more than 350 years.

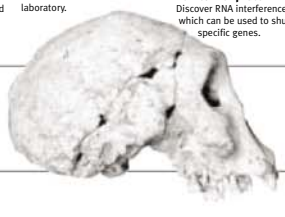
1995
Bose-Einstein condensate—a new form of matter— created in laboratory.

1995
Suo Guo and Kenneth Kemphues
Discover RNA interference, which can be used to shut off specific genes.

1997
Ian Wilmut and Keith Campbell →
Clone a sheep from adult cells.
In the spring of 1997, Ian Wilmut and Keith Campbell introduced the world to the first cloned mammal—a sheep named Dolly. Scientists then cloned pigs in hopes of using them as organ factories for humans; other cloned organisms include frogs, mice, and more.

2001
Chinese farmers find feather-covered, duck-like dinosaur fossil.
When Chinese farmers found a fossil of a duck-like dinosaur in 2001, some scientists said it was covered in feathers, but others disagreed. Yet, many dinosaur fossils—from one of a Velociraptor attacking a Protoceratops to a Tyrannosaurus rex named Sue—stir controversy when reconstructing the past.

2001
Toumaï fossil, skull of oldest known human relative, discovered. →



2003
Jie Han and Pieter Jonker
Invent theory of fault-tolerant nanocircuits that lets these microscopic networks work even when they contain defects.

2004
Two rovers explore opposite sides of Mars for a year and detect evidence of its wet past.

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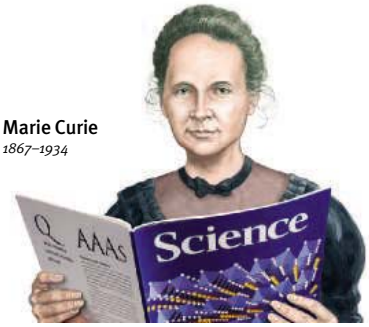

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1867–1934

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WASHINGTON STATE UNIVERSITY

ASSISTANT/ASSOCIATE/FULL PROFESSOR
Neurobiology of Substance Abuse

The Department of Veterinary and Comparative Anatomy, Pharmacology, and Physiology (VCAPP) at Washington State University invites applications for a tenure-track position to begin July 1, 2006, or earlier. Required qualifications: Ph.D., Ph.D./D.V.M., Ph.D./M.D., or equivalent degree in an area related to neurobiology of substance abuse; at least two years postdoctoral training and a record of excellence in research. Applicants will be expected to establish an outstanding independent research program that will attract continued extramural funding and to participate in the teaching mission of the Department. He/she should have excellent communication skills and will be expected to teach undergraduate, graduate, and/or veterinary students. Willingness and ability to engage in collaborative research and graduate training within a vibrant local neuroscience community are also desired. Screening of application materials will begin August 15, 2005. The application must include a cover letter, curriculum vitae, description of teaching experience and philosophy, summary of research interests and goals, and the names, e-mail addresses, and contact information for three references. Indicate the rank sought. Send application materials to:

Neurobiology Search Committee
Department of VCAPP
Washington State University
Pullman, WA 99164-6520
E-mail: bmorton@vetmed.wsu.edu

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UNIVERSITY OF Nebraska Medical Center

FACULTY POSITION, BIOCHEMISTRY AND MOLECULAR BIOLOGY
University of Nebraska Medical Center

The Department of Biochemistry and Molecular Biology invites applications for two tenure-track positions at the rank of **ASSISTANT/ASSOCIATE PROFESSOR.** Qualifications include a Ph.D. or M.D. degree and relevant postdoctoral experience. Preference for both positions will be given to outstanding candidates who can utilize state-of-the-art approaches in biochemical and molecular approaches to cancer research. One position will be held for an individual skilled in application of proteomic analysis. The ideal candidates will have research interests and experience that will supplement ongoing research in the Department (**website: http://www.unmc.edu/Biochemistry/**). To qualify for an Associate Professor position, extramural (NIH or equivalent) funding is required with a significant record of publication in the field.

The successful applicant will be expected to have or develop a funded, independent research program and to contribute significantly to the teaching programs of the Department. Submit your curriculum vitae, a description of research interests and teaching experience, and three letters of reference to: **Dr. Surinder K. Batra, Chair, BMB Search Committee, Department of Biochemistry/Molecular Biology, 985870 Nebraska Medical Center, Omaha, NE 68198-5870.** Also e-mail a PDF or MS Word file of the above documents to **e-mail: biochem@unmc.edu.** *The University of Nebraska Medical Center is an Equal Opportunity/Affirmative Action Employer. Minorities and women are encouraged to apply.*

THE UNIVERSITY OF TEXAS SOUTHWESTERN MEDICAL CENTER

ASSISTANT/ASSOCIATE PROFESSORS: The Department of Physiology invites outstanding scientists with Ph.D., M.D., or equivalent degrees to apply for several faculty positions as tenure-track Assistant or Associate Professors. Candidates who use integrative and innovative molecular, genetic, biophysical, biochemical, and systems biology approaches to analyze important biological problems are encouraged to apply. The scientific excellence of the candidates is more important than the specific area of research.

These positions are part of the continuing growth of the Department at one of the country's leading academic medical centers and will be supported by significant laboratory space, competitive salaries, and exceptional startup packages. The UT Southwestern faculty, which includes four Nobel Prize laureates, 15 members of the National Academy of Sciences (NAS), and 17 members of the NAS Institute of Medicine, conducts more than 2,000 research projects that are supported by $300 million grant funding annually.

Applicants should submit curriculum vitae, a brief statement of research plans, and arrange to have three letters of reference sent to: **Helen L. Yin, Ph.D., Chair of Search Committee, c/o Gena McElyea, Department of Physiology, The University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75390-9040.** *UT Southwestern strongly encourages applications from women, minorities, and people with disabilities. An Affirmative Action/Equal Opportunity Employer.*

POSTDOCTORAL POSITIONS immediately available to study the molecular pathogenesis of bladder cancer, renal stone disease, and urinary tract infection. Applicants with Ph.D. and/or M.D. and strong background in molecular biology and commitment to basic research are encouraged to send curricula vitae to: **Dr. Xue-Ru Wu, Department of Urology, New York University School of Medicine (e-mail: xue-ru.wu@med.nyu.edu).**

Tenure-Track Investigator Membrane Protein Biochemistry

The Intramural Research Program at NINDS invites applications to fill a faculty position at the level of tenure-track investigator in the area of eukaryotic membrane protein biochemistry and structure. We seek a creative, interactive scientist to establish an independent research program focused on mechanistic and structural investigations of eukaryotic membrane proteins, with a particular emphasis on solving problems associated with the high level production, purification and stability of eukaryotic membrane proteins. Candidates working on G-protein coupled receptors are particularly encouraged to apply. A PhD and at least four years postdoctoral experience are required. The position includes salary, a competitive start-up package, as well as an annual operating budget to support the research program. Laboratory space will be provided in the 5625 Fishers Lane research facility in Rockville, MD, which is located several miles from the main NIH campus in Bethesda, MD. Applicants should send curriculum vitae including bibliography, statement of research interests and the names of three references to: **Story Landis, PhD, Director, NINDS, NIH, c/o Peggy Rollins, Building 35, Room GA908, 35 Convent Drive, MSC-3716, Bethesda, MD 20892-3716. Applications must be postmarked by August 31, 2005.**

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Tenure-Track Position

With nation-wide responsibility for improving the health and well being of all Americans, the Department of Health and Human Services (DHHS) oversees the biomedical research programs of the National Institutes of Health (NIH) and those of NIH's research Institutes.

The National Institute of Mental Health (NIMH), a major research component of the NIH, and the DHHS, is recruiting for a tenure-track neuroscientist in the new Genes, Cognition and Psychosis Program (GCAP). With a complimentary budget and staff, the individual selected for this position will be expected to establish an independent research program focused on cellular and molecular neuroscience relevant to schizophrenia.

The successful individual must possess an M.D. and/or Ph.D. degree, and experience in cellular biochemistry, imaging, or electrophysiology is preferable. At least five years of relevant research experience is required.

There are strong interactions among the independent research group, and the position offers unparallel opportunities for interdisciplinary collaboration with the scientists within GCAP and throughout the NIH. Salary is commensurate with experience and accomplishments, and a full Civil Service package of benefits (including retirement, health, life, and long term care insurance, Thrift Savings Plan participation, etc.) is available.

The strong scientific environment and outstanding equipment resources at NIH makes this a unique opportunity for an outstanding scientist. Interested candidates should send curriculum vitae, statement of research interests, accomplishments and future goals, and three letters of recommendation to the **Chair, Search Committee for a Tenure Track Investigator in the area of Cell Biology, National Institute of Mental Health, Building 10, Room 4N-222, 9000 Rockville Pike, Bethesda, MD 20892, or by email to: steyerm@mail.nih.gov by July 31, 2005.**

Postdoctoral Training Positions Translational Cancer Genomics

The Oncogenomics Section, at the National Cancer Institute, NIH, has two post-doctoral training positions available in translational genomics. Ongoing research efforts involve genomic and proteomic approaches to the investigation of Neuroblastoma. Using these approaches we have identified prognostic markers and potential new therapeutic targets in cancers. The candidate will work on combining molecular and genomic approaches to validate these targets both in vitro and animal models to bring these reagents to the clinic. The candidate should hold either a Ph.D. or M.D. degree and have an interest in oncology and significant experience in genomics and the microarray technology. Experience in mouse/animal models is preferred. Candidates should have less than five years post-doctoral experience.

Correspondence, names of references and CV should be sent to **Dr. Javed Khan, Advanced Technology Center, National Cancer Institute, Room 225B, 8717 Grovemont Circle, Gaithersburg, MD 20877, or via email at khanjav@mail.nih.gov**

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Roundtable Discussion Among the 2004 Pioneer Awardees

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NIDDK

NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

Postdoctoral Fellowships In Signaling Pathways And Disease

Postdoctoral Fellowships are available in the Metabolic Diseases Branch, NIDDK, NIH. The Branch is similar to a small academic department and has excellent laboratory facilities. The intramural program of the NIH offers an outstanding research environment and has been rated by *The Scientist* as one of the top places for Postdocs to work. The Branch is located on the main intramural campus of the NIH in Bethesda, Maryland, a 20-minute ride from Washington, D.C. Applications are invited from individuals of the highest caliber who have obtained a Ph.D., or M.D. degree within the last 5 years. Positions are available in the following areas:

Regulation of *GNAS* imprinting and role of the stimulatory G protein $G_s\alpha$ in metabolic regulation

Postdoctoral positions are available to study the mechanisms of *GNAS* and $G_s\alpha$ imprinting in mouse models and in patients with *GNAS* imprinting defects. We are also studying the role of $G_s\alpha$ and other *GNAS* gene products in metabolic regulation using several germline and tissue-specific *GNAS* knockout mouse models **(Lee S. Weinstein, MD, leew@amb.niddk.nih.gov)**

Function of tumor suppressor *HRPT2*

A postdoctoral position is available to study the novel tumor suppressor gene, *HRPT2*, recently implicated in parathyroid cancer and the familial cancer syndrome HPT-JT (hyperparathyroidism-jaw tumor syndrome). We are studying parafibromin, the protein encoded by *HRPT2*, in both mammalian and invertebrate models systems with the aim of elucidating the biological pathway(s) critical for its tumor suppressor function. A strong background in molecular biology and/or signal transduction is required. **(William F. Simonds, MD, wfs@helix.nih.gov)**

Applicants must have an advanced degree (M.D., Ph.D., or equivalent). Salary and benefits will be commensurate with the experience of the applicant. **Interested candidates should send a letter stating their interests, their curriculum vitae, list of publications, and the names of three references to the appropriate individual by e-mail or to Building 10, Room 8C-101; 10 Center Dr MSC 1752; National Institutes of Health; Bethesda, MD 20892-1752.**

hope ON THE HORIZON

Assistant/ Associate Professor

Department of Gene Regulation & Drug Discovery
Beckman Research Institute
The City of Hope National Medical Center

The mission of this newly established department is to integrate approaches in gene regulation, chemistry, and emerging molecular technologies. We invite applications for a faculty position at the Assistant or Associate Professor level from candidates whose research interests include the study of molecular pathways and the development of small molecule therapies for atherosclerosis, obesity, diabetes and related disorders. Appointed faculty will participate in the Graduate School of Biological Sciences.

The Beckman Research Institute provides an environment that encourages interdisciplinary, collaborative interactions with institutional programs in molecular biology and gene regulation, immunology, diabetes and endocrinology, cancer-related drug discovery, hematology and bone marrow transplantation. Our rich set of core resources include chemical libraries and high-throughput screening, transgenic mouse production, microarray-analysis, mass spectrometry, NMR, cell-sorting, microscopic imaging, oligonucleotide and peptide synthesis, DNA and peptide sequencing, biomedical informatics, pathology, and others. For further information visit our Web site at: **http://www.cityofhope.org/GeRDD.**

Requirements include a Ph.D. degree, postdoctoral experience, and the potential to establish, or to have established, an independent and creative research program. Full-time salary support is available (institutionally-funded) for this tenure-track position, as are extremely competitive start-up packages.

Please submit (in MS Word or PDF format only), a curriculum vitae, one-page summary of past research accomplishments, one-page summary of future research directions, and the names, addresses, e-mail addresses, and telephone numbers of at least three references to: **GeRDD Search Committee, GeRDDsearch@bricoh.edu, Ph: 626-256-4673, ext. 62012.** In addition, please request that your references email their letters as above. Completed applications (including letters of reference) will be evaluated immediately, and will be considered until mid-January 2006 or until a recruit has been identified.

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We seek an investigator whose established research program(s) at the interface between advanced technology and human and environmental health will complement the existing strengths of our faculty. We are particularly interested in candidates whose research, interdisciplinary interests, vision, and leadership qualities will result in the creation of a world-class nanobiotechnology research and graduate education center that will leverage high-level collaborations with OHSU's research and patient care communities as well as with other institutions in Oregon.

Qualified applicants are encouraged to submit a letter of application, a curriculum vitae, and a summary of research and educational objectives to:

Dr. William H. Glaze, Associate Dean
Gordon and Betty Moore Chair Search Committee
OGI School of Science and Engineering
Oregon Health and Science University
20000 NW Walker Road, Mail Code OGI-801
Beaverton, OR 97006-8921

Electronic submissions may be sent to: **hendricc@ohsu.edu**

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Ph.D. Level Scientist with 5 yrs. protein purification development experience in biotech. Req. GLP/GMP experience, hands-on, proficient in purification techniques bench to production scale.

Senior Research Associate
BS/MS level Scientist, cell biology/biology 2-5 yrs. experience in assay design and performance. GLP/GMP experience desirable, hands-on with current technology experience required.

Receptor BioLogix Inc., based in South San Francisco, is a biopharmaceutical company focused on developing a newly discovered class of protein therapeutics to treat cancer and autoimmune diseases. Receptor BioLogix's lead clinical product is Dimercept™, a broad-spectrum anti-cancer agent that represents a new class of receptor modulators called Intron Fusion Proteins™.

The successful candidates will join a strong group of molecular and cellular biologists.

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db@receptorbiologix.com

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Health Research in a Changing World

Fighting Diseases and Improving Lives

DEPARTMENT OF HEALTH AND HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

With nationwide responsibility for improving the health and well-being of all Americans, the National Institutes of Health (NIH) and the National Institute of Allergy and Infectious Diseases (NIAID) conducts and supports a global program of research aimed at improving diagnosis, treatment and prevention of immunologic, allergic and emerging infectious diseases. NIAID's mission is driven by a strong commitment to basic research, which incorporates the complementary fields of vaccine research, immunology, microbiology and infectious diseases.

NIAID is a world leader in medical research and is on the cutting edge of medical and scientific research/discovery, including areas of biodefense (i.e., smallpox and anthrax), HIV/AIDS research worldwide, vaccine research (i.e., Ebola), asthma and allergic diseases and other advances of medical and scientific research. NIAID has state-of-the-art equipment, facilities and laboratories and a highly trained staff with the potential and desire to find a cure/vaccine for some of the deadliest infectious diseases known to mankind.

The NIAID is committed to maintaining its stature as a premier research institution by building an inclusive workforce through the Workplace Diversity Initiative and Affirmative Action programs. The NIAID's commitment to equal opportunity and diversity in recruiting, hiring and career development will help ensure the continued output of excellent science.

Are you ready for an exciting career that could help improve millions of lives around the world? Then consider joining the scientific and medical forces at NIAID. The NIAID, NIH, DHHS offers competitive salaries and a comprehensive benefits package. Most job opportunities are conveniently located in Bethesda, Maryland within minutes of thousands of cultural, recreational, entertainment and educational centers. Positions are also available in Hamilton, Montana.

We invite you to explore job opportunities and submit your resumes online at: http://healthresearch.niaid.nih.gov/science. Whether you are a scientist, nurse, health specialist or have a background in other support disciplines such as finance, communications and administration, your individual talents are needed to support our mission. Do not delay. Medical history won't wait. NIAID is currently searching for qualified Medical Officers, Nurse Consultants, Scientific Review Administrators, Senior Regulatory Affairs Specialists and Health Specialists.

We are happy to respond to your questions, and you may contact us toll-free at 888-798-4991.

Please visit us at the Drug Discovery conference 8/7-12/05 at Booth #539
Please reference "Science" on your resume.

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Department of Health and Human Services
National Institutes of Health
National Institute of Allergy and Infectious Diseases
Proud to be Equal Opportunity Employers

Endowed Chair
Pediatric Cardiovascular Research
Scott & White Health System
Texas A&M University System Health Science Center
College of Medicine

The **Children's Hospital at Scott & White** and **The Texas A&M University System Health Science Center College of Medicine** are seeking a nationally recognized research scientist as the first holder of the Josephine Ballard Endowed Chair in pediatric cardiovascular research. Applicants should be accomplished investigators (Ph.D., M.D. or M.D./Ph.D.) at the associate or professor level with current federal grants and a proven track record in cardiovascular basic, clinical, and/or translational research. The successful candidate will join an expanding faculty within a large academic healthcare system. The chair holder will play a critical role in directing and expanding research activities in pediatric cardiovascular disease, in close collaboration with investigators in the Cardiovascular Research Institute and other local, national and international experts in cell biology, genomics and proteomics.

The Children's Hospital at Scott & White serves a large clinical base throughout Central Texas. There are outstanding clinical practice and laboratory facilities on campus that perform state of the art molecular and cellular biology techniques, flow cytometry, proteomics and genomics as well as biostatistical support services. Animal laboratory facilities include areas to perform medical and surgical procedures. Laboratory space and an appropriate start-up package for the chair holder will be provided. The Scott & White Healthcare system is one of the largest multi-specialty integrated delivery systems in the nation. Scott & White is the primary clinical and hospital teaching campus for the College of Medicine. Academic appointments at the associate and professor level through the College of Medicine are commensurate with qualifications and experience.

Interested candidates should send a copy of their curriculum vitae, letter addressing their qualifications and a list of 3 individuals who can provide references to: **Don P. Wilson, M.D., Chair, Search Committee for Josephine Ballard Centennial Chair in Pediatric Cardiovascular Research; Chairman, Department of Pediatrics, 2401 South 31st Street, Temple, Texas 76508, 254-724-4363, fax 254-724-1938, email: dwilson@swmail.sw.org**

Scott & White is an equal opportunity employer. For more information regarding Scott & White and The Texas A&M University System Health Science Center College of Medicine, please log onto: www.tamu.edu and www.sw.org.

Laboratory Director Position in RIKEN, Japan

RIKEN invites applications for the position of Laboratory Director (Full Professor Level) to lead a new laboratory in Harima Institute. Candidates should have a strong research record in structure-based materials science using synchrotron hard x-rays, established international reputation, and an ardent interest in extending his/her research activities to interdisciplinary fields. The successful candidate will be responsible for the laboratory's overall management and research strategy, directing research projects, and contributing to more general aspects of the institute's management and research planning activities. The post is a permanent appointment, subject to RIKEN's mandatory retirement at age of 60. Terms and conditions of employment shall include a director-level salary and be in accordance with RIKEN's procedures for appointing Laboratory Director. The successful applicant will be expected to take up this position from April 1st, 2006.

An applicant should send: a full curriculum vitae with photograph; a list of publications with one copy each of five key publications; a statement explaining research experience, reasons for his/her application, proposals for research at RIKEN (these should not exceed five pages); and the names and addresses of three referees. All applications are to be received by September 30, 2005.

Address materials to: Dr. Tetsuya Ishikawa, Head of the Laboratory Director Nominating Committee, Harima Institute, RIKEN (The Institute of Physical and Chemical Research), 1-1-1 Kouto, Mikazuki, Sayo-Gun, Hyogo 679-5148, JAPAN
E-mail address: ishikawa@spring8.or.jp

Information about RIKEN is available on Web Site (http://www.riken.go.jp/)

NYCOM NYIT

NEW YORK COLLEGE OF OSTEOPATHIC MEDICINE
OF NEW YORK INSTITUTE OF TECHNOLOGY

The New York College of Osteopathic Medicine of New York Institute of Technology (NYCOM), the only osteopathic medical school in New York State, invites applications for the following position:

FACULTY POSITIONS – BIOMEDICAL SCIENCES

The New York College of Osteopathic Medicine of the New York Institute of Technology has academic positions available in Physiology, Pathology, Pharmacology/Toxicology, and Cellular/Molecular Biology at the Assistant/Associate Professor level. We are seeking individuals with the ability to establish and sustain vigorous and creative independent and/or collaborative research programs in their area of interest. Other responsibilities will include teaching first and second year medical students in an innovative medical school curriculum. Demonstrated excellence in teaching is highly desirable. Participating in select departmental and college committees will be required. Faculty rank and salary will be commensurate with experience. Minimal qualifications include a Ph.D. and/or D.O. or M.D. degree, plus 2-3 years of post-doctoral research experience or relevant clinical experience. Consideration of applicants will begin immediately and will continue until the positions are filled. Anticipated start date will be August/Sept. 2005 or soon thereafter. Interested candidates should send their CV, recent reprints, statement of research interests, and arrange to have 3 letters of reference sent to: Charles Pavia, Ph.D., Chairman, Search Committee, Dept. of Biomedical Sciences, NYCOM of NYIT, Northern Boulevard, P.O. Box 8000, Old Westbury, NY 11568. NYIT is an AA/EEO institution.

www.nyit.edu

Department of Bioengineering
Faculty Positions

The Department of Bioengineering at the University of Pennsylvania has two open standing faculty positions at any rank. We are considering candidates with superb academic credentials that hold exceptional promise for a career in bioengineering research. A commitment to teaching and service should also be apparent. We will consider candidates in all fields of bioengineering, including but not limited to: large scale/systems biology (including proteomics and genomics), drug delivery, cell and tissue engineering, neuroengineering, novel bioengineering devices and biomaterials, and imaging. We will consider candidates who perform basic research, translational research, or the combination.

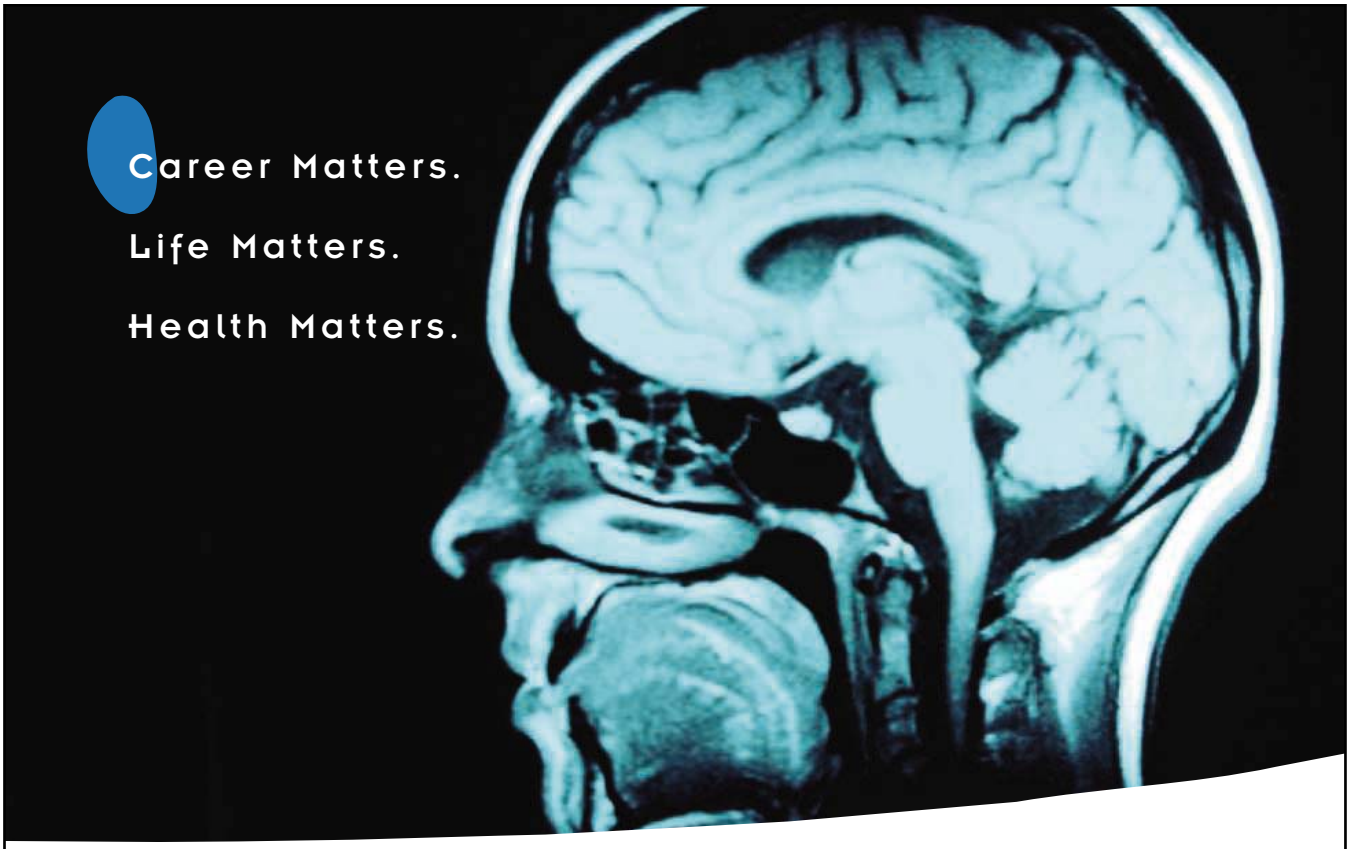
To apply, please send a CV, a statement of research interests, a statement of teaching interests, and a list of five references, in hard copy, by December 1, 2005 to:

Please submit curriculum vitae and a brief statement of research interests by June 30, 2005 to:

Daniel A. Hammer
Ennis Professor and Chair
Department of Bioengineering
University of Pennsylvania
120 Hayden Hall
3320 Smith Walk
Philadelphia, PA 19104
(215) 573-6761

The University of Pennsylvania is an affirmative action/equal opportunity employer and is strongly committed to diversity. Minorities/Females/Individuals with Disabilities/Veterans are encouraged to apply.

ScienceCareers.org



CAREER OPPORTUNITIES

As a global leader in pharmaceutical drug discovery, sanofi-aventis' goal is to treat people with the very best therapeutic agents - *because health matters.* Our strong development pipeline is based upon scientific and medical innovation. We have a major commitment to CNS, our largest therapeutic department.

The **CNS** division is **expanding** its **psychiatric** and **neurological** disease areas. We currently have multiple career opportunities for innovative scientists at the **BS, MS and PhD** levels at our **Bridgewater, NJ location.**

Psychopharmacology: Focus on Schizophrenia

Job Code: SMA10442

Development and implementation of rodent and primate models of working memory and attention

Neurological Diseases: Focus on Multiple Sclerosis

Job Code: SMA10659

Development and implementation of in vivo and in vitro models of inflammation and repair

Molecular and Functional Neuropharmacology: Focus on Lead Identification and Optimization

Job Code: SMA10651

Development and implementation of biochemical and molecular assays for *in vitro* and *in vivo* pharmacology, in vivo proof of mechanism, and acute pharmacodynamics

At the heart of all that matters are people. Connected in purpose by career, life, and health. We at Sanofi-Synthelabo and Aventis Pharmaceuticals, members of the sanofi-aventis Group, are working together to focus on what is essential to us all–health.

Driven by a pioneering spirit, a strong set of core values and a mosaic of talent worldwide, we strive for success–in health. In doing so, we strengthen careers and enrich lives.

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By embracing diversity of thought and culture, sanofi-aventis fosters positive, innovative thinking that will benefit people worldwide.

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Because health matters

Singapore Bioimaging Consortium

A*STAR

The Singapore Bioimaging Consortium (SBIC) is a new initiative by A*STAR that aims to use and develop state-of-the-art imaging technologies to pursue research leading to useful clinical applications. Prof. Sir George Radda is the Chairman of the newly formed Singapore Bioimaging Consortium (SBIC). It brings together Scientists and Researchers from the universities, hospitals and commercial laboratories in an integrated, cutting-edge programme. Four technology platforms have been identified for core research programmes: optical imaging, image processing and management, small animal imaging with Magnetic Resonance (MRI and MRS) and the development of chemical/biological probes.

POST-DOCTORAL
LABORATORY OF METABOLIC MEDICINE

We are inviting enthusiastic Researchers to join us in working in metabolic medicine especially in the area of diabetes and obesity and the role of the central nervous system in these conditions. Focus will be on mouse models of human disease. Expert assistance in animal studies will be available. The research will be conducted in a laboratory equipped with advanced facilities for conducting physiological, biochemical and cellular studies in close association with state-of-the-art imaging facilities.

Job Requirements:

- A PhD in Cell or Molecular Biology, Biochemistry, Physiology or related field
- Enthusiasm for research in the field of diabetes/obesity and metabolic medicine
- Experience in whole animal investigations, i.e. physiological and biochemical techniques, mammalian cell culture and some knowledge of transgenic animal work is an advantage but not essential
- Publications in International Refereed Journals

An attractive and competitive remuneration package is offered. This includes housing subsidy, a settling-in allowance, as well as air passage for staff and family, for the successful candidate who is either a foreigner or Singapore citizen who has worked continuously abroad for over 5 years.

Please send a full CV, with names and contact details of two academic referees, to **Dr Ngee-Chih Foo** at **Foo_Ngee_Chih@a-star.edu.sg**.

The closing date for this application is **1 August 2005**.

Enquiries on other research positions are welcome. Visit our website: www.a-star.edu.sg for more details.

The **Department of Radiology at the University of California, San Francisco** is seeking two faculty members at the **Assistant or Associate Professor** rank for the In Residence or Adjunct series to mount exciting research programs in the development of high field magnetic resonance imaging and spectroscopy techniques and to teach graduate, professional, and postdoctoral students. Incumbents will be expected to participate in the UCSF/UCB Joint Graduate Group in Bioengineering and the California Institute for Quantitative Biomedical Research (QB3), which is a joint program of the UC campuses at San Francisco, Berkeley, and Santa Cruz.

Applicants should have a doctoral degree or equivalent in biological, engineering or physical sciences, with a major focus on the development and applications of biomedical imaging using whole body 3T and 7T MR scanners. Specific areas of interest include, but are not limited to: The development of fMRI and other imaging techniques for studying cognitive function and In vivo multi-nuclear MRS with an emphasis on applications to cancer. Applicants must submit curriculum vitae, one or two key publications, two-page summary of past research and future goals, and must have three letters of reference sent to the Search Committee. Electronic submission of all materials is greatly preferred. **Daniel B. Vigneron, PhD, Chair, Radiology Search Committee, Department of Radiology Box 2512, University of California San Francisco, San Francisco, CA 94143-2512**. Electronic submissions should be sent to: **Marisa.Thorne@radiology.ucsf.edu**.

The University is an Equal Opportunity/ Affirmative Action Employer. All qualified applicants are encouraged to apply, including minorities and women.

MORGAN STATE UNIVERSITY
Assistant or Associate Professor of Microbiology, Ecology and Toxicology (Tenure Track)

Positions: The Biology Department at Morgan State University, Baltimore, Maryland, is seeking to fill faculty positions in Microbiology, Ecology and Toxicology for the 2005-2006 academic year. The positions are tenure track at the Assistant or Associate Professor level.

Qualifications: Potential candidates must possess a Ph.D. degree and postdoctoral training. Candidates must demonstrate ability to teach courses in the discipline or related course(s) at the undergraduate and graduate levels. Also candidates will be expected to participate in the training of graduate students under the rubric of the Bioenvironmental Science Ph.D. Program. Additionally, successful applicants will be expected to develop and obtain extramural funding for strong research programs relevant to the environmental sciences.

University: Morgan State is a historically black institution with the unique designation as Maryland's public urban university. As an urban university, Morgan serves an ethnically and culturally diverse student body with an enrollment of 7,000. More detailed information about the Department and the University can be obtained from our website: www.morgan.edu.

Department: The Biology Department is a multi-discipline unit consisting of 32 full-time faculty members. There are more than 400 undergraduate majors and approximately 30 graduate students. The department offers a Bachelor of Science Degree and a Masters of Science (Biology) Degree. In addition the department offers an interdisciplinary Ph.D. Degree in Bioenvironmental Science.

To Apply: Please submit a letter of interest, curriculum vitae, teaching philosophy, copies of official transcripts, and three letters of recommendation to: **Dr. Juarine Stewart, Dean, School of Computer, Mathematical and Natural Sciences, Morgan State University, 1700 East Cold Spring Lane, Baltimore, Maryland 21251**.

Closing Date: The review of applications will begin immediately and will continue until position is filled.

EQUAL OPPORTUNITY/ AFFIRMATIVE ACTION EMPLOYER (EEO/AA).

University of Massachusetts Amherst

ASSISTANT PROFESSOR
Genomic Biology

The Department of Microbiology at the University of Massachusetts, Amherst invites applications for a tenure-track position at the **Assistant Professor** level. The Department is seeking outstanding candidates in the area of Computational Biology, Bioinformatics and or Systems Biology to investigate biological problems in medical or environmental microbiology involving prokaryotes, eukaryotes and or viruses. Candidates must have a strong commitment to excellence in undergraduate and graduate education. The successful candidate will be expected to establish a strong independent, extramurally funded research program and participate in the teaching of undergraduate and graduate courses. Research facilities and competitive salary and start-up funds will be provided. Opportunities exist to develop strong collaborations with faculty in the Five College Consortium and at Bay State Medical Center. Ph.D. in Biochemistry, Molecular Genetics, Computer Science or a related field is required.

Review of applications will begin **August 5, 2005** and continue until the position is filled. Applicants should send a letter of application, curriculum vitae, a summary of research interests and future plans, teaching interests and representative publications electronically as PDF files to the following address: **microbio-dept@microbio.umass.edu**. Arrange to have three letters of recommendation sent to: **Microbiology Search Committee Chair, Department of Microbiology, 203 Morrill Science Center IV North, 639 North Pleasant Street, University of Massachusetts, Amherst, MA 01003**.

The University of Massachusetts is an Affirmative Action/Equal Opportunity Employer. Women and members of minority groups are encouraged to apply.

ARGONNE NATIONAL LABORATORY

The Argonne National Laboratory Named Postdoctoral Fellowship Program

The Director's Office initiated these special postdoctoral fellowships at Argonne National Laboratory, to be awarded internationally on an annual basis to outstanding doctoral scientists and engineers who are at early points in promising careers. The fellowships are named after scientific and technical luminaries who have been associated with the Laboratory and its predecessors, and the University of Chicago, since the 1940's.

Candidates for these fellowships must display superb ability in scientific or engineering research, and must show definite promise of becoming outstanding leaders in the research they pursue; the Laboratory intends to award four such fellowships this coming year. Fellowships are awarded for a two-year term, with a possible renewal for a third year, and carry a stipend of $71,000 per annum with an additional allocation of up to $20,000 per annum for research support and travel.

Requirements for Applying for an Argonne Named Postdoctoral Fellowship:

The following documents must be sent **via e-mail to: fellowships@anl.gov by October 14, 2005**.

- Letter of Nomination (Recommendation from individual who supports your candidacy for the fellowship.)
- Curriculum Vitae (Include the names of the Nominator and two additional references.)
- Two letters of reference (It is the candidate's responsibility to arrange that the two reference letters be sent to the Laboratory **via e-mail** prior to the **October 14, 2005 deadline.)**
- Bibliography of publications
- Bibliography of preprints
- Description of research interests to be pursued at the Laboratory (We encourage applicants to contact Argonne staff in their areas of interest in order to explore possible areas of research.)
- Name of Argonne Division(s) in which you would like to work

All correspondence should be addressed to ANL Named Postdoctoral Fellowship Program. One application is sufficient to be considered for all named fellowships. For additional details, visit the Argonne web site at **http://www.anl.gov**. Argonne is an equal opportunity employer.

Argonne is operated by The University of Chicago for the U.S. Department of Energy Office of Science.

Computational Chemistry and Biology Opportunities at D. E. Shaw Research and Development

Extraordinarily gifted computational chemists, biologists, and other computational scientists are sought to join a rapidly growing New York-based research group that is pursuing an ambitious, long-term strategy aimed at fundamentally transforming the process of drug discovery.

Candidates should have world-class credentials in computational chemistry, biology, or physics, or in a relevant area of computer science or applied mathematics, and must have unusually strong research skills. Relevant areas of experience might include protein structure prediction, the computation of protein-ligand binding affinities, the study of biologically important systems using molecular dynamics and/or Monte Carlo simulation, and the application of statistical mechanics to biomolecular systems—but specific knowledge of any of these areas is less critical than exceptional intellectual ability and a demonstrated track record of achievement. Current areas of interest within the group include molecular dynamics simulation of functionally significant globular and membrane proteins, the prediction of protein structures and binding free energies, structure- and ligand-based drug design, characterization of protein-protein, protein-nucleic acid and protein-lipid interactions, and the development of algorithms for biomolecular simulations.

This research effort is being financed by the D. E. Shaw group, an investment and technology development firm with approximately $15 billion in aggregate capital. The project was initiated by the firm's founder, Dr. David E. Shaw, and operates under his direct scientific leadership.

We are eager to add both senior- and junior-level members to our world-class team, and are prepared to offer above-market compensation to candidates of truly exceptional ability. Please send your CV (including list of publications, thesis topic, and advisor, if applicable) to sciencemag-cc@desrad.deshaw.com.

D. E. Shaw Research and Development, L.L.C. does not discriminate in employment matters on the basis of race, color, religion, gender, national origin, age, military service eligibility, veteran status, sexual orientation, marital status, disability, or any other protected class.

D E Shaw & Co

Improving Lives

Group Leader/Sr. Group Leader

This individual will play a key role as part of a multi-disciplinary team of scientists focused on identifying new cancer therapies. Primary focus is to determine efficacy of promising novel oncology agents using *in vivo* tumor models. Candidate will supervise Ph.D. and non-Ph.D. scientists, and manage several projects simultaneously. Candidate will develop new orthotopic & metastasis models and participate in mechanism of action studies using IHC, imaging, biomarker and cell biology techniques. Candidates will also interact with the clinical development team.

PhD is required with 6+ years R&D and drug discovery experience. At least 3 years *in vivo* experience and a minimum of 2 years prior people/project management is also required. Strong scientific background with an extensive peer reviewed publication record. Preference will be given to candidates with prior cancer research experience.

To apply, please visit: www.abbott.com. Click Career Center, Job Search, Job Opportunities, Search Openings, Enter **28325BR** into the Keyword field.

Follow your aspirations to Abbott for diverse opportunities, competitive salaries, great benefits, a 401(k) retirement savings plan, a company paid pension plan and profit sharing, all with a company providing the growth and strength to build your future.

Abbott welcomes and encourages diversity in our workforce. EEO/AA.

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A Promise for Life

FACULTY POSITION ANNOUNCEMENT Assistant Professor: Mammary Gland Biologist, Department of Animal Science, University of California, Davis

The Department of Animal Science in the College of Agricultural and Environmental Sciences seeks applicants for an Assistant Professor in Mammary Gland Biology with teaching, research and outreach responsibilities consistent with the mission of the California Agricultural Experiment Station. We seek outstanding applicants with a Ph.D. or equivalent degree. Post-doctoral experience is preferred. These educational experiences should have emphasized mammary gland biology and prepared the candidate for the study of mammary gland biology using cutting-edge biotechnological approaches such as functional genomics, proteomics, or metabolomics. Appointees are expected to develop an extramurally funded research program emphasizing mammary gland function. The appointee is required to teach an undergraduate course in the biology of lactation. Additional contributions to departmental courses and graduate education will be expected. Mentoring of graduate students, student advising, participation in outreach programs, curricular development, and performance of University service are also expected. The successful candidate is expected to develop a research and teaching program relevant to the California dairy industry, the state's largest industry, and contribute to outreach consistent with the missions of the Agricultural Experiment Station. Positions are nine-month tenure track appointments; eleven-month term employment to be offered and continued based upon academic personnel review. The position will be available on or about November 1, 2005.

Applicants should submit a CV including list of publications, transcripts, a detailed description of research and teaching accomplishments, and statement of future plans, copies of relevant in-press publications and manuscripts, and the names and contact information of three to five references to: **Professor A.M. Oberbauer, Search Committee Chair, Department of Animal Science, One Shields Avenue, University of California, Davis, CA 95616**, telephone **(530) 752-4997**, **amoberbauer@ucdavis.edu**. Open until filled but to ensure consideration, applications should be received by **August 31, 2005**. A more detailed job description is available at **http://animalscience.ucdavis.edu/positions**. E-mail applications will not be considered.

The College of Agricultural and Environmental Sciences and Department of Animal Science at UC Davis are committed to building a diverse faculty, staff and student body reflecting the population and educational needs of California and the nation.

Two Postdoctoral Positions Department of Pharmaceutical Sciences University of Maryland, Baltimore

Dr Michael Shapiro has several new projects available, including: (1) Application and development of NMR methodologies and strategies and their corresponding utility to understand the structure and function of therapeutic proteins in structure-based drug design programs - of particular interest are the topics of conformational selection and adaptive proteins; (2) Protein NMR chemical shifts and relaxation parameters to understand ligand-protein interactions; (3) NMR and biophysical methods in drug discovery using fragment based drug design; (4) Metabanomics using NMR spectroscopy.

The ideal candidates will have extensive experience in Protein NMR spectroscopy, protein biochemistry and molecular biology. Motivated individuals with broad interests in the area of biomolecular NMR in drug design. The university is well equipped with Bruker 800 and 600 MHz spectrometers with cryoprobes and a Varian 500 MHz system.

The Department of Pharmaceutical Sciences of the University of Maryland is part of the Health Science Centers of the University of Maryland (**http://www.pharmacy.umaryland.edu/graduate/psc/**) and is located in downtown Baltimore. The campus is within walking distance of Baltimore's Inner Harbor and Camden Yards, and is approximately one hour from Washington, DC. The University of Maryland is an equal opportunity employer and salary and benefits are competitive and commensurate with experience.

Individuals interested in either position should send their curriculum vitae and three letters of recommendation to:

Dr. Michael Shapiro
Department of Pharmaceutical Sciences
School of Pharmacy
University of Maryland
20 Penn Street
Baltimore, MD 21201

mshapiro@rx.umaryland.edu
410-706-0886

THE UNIVERSITY OF TEXAS AT AUSTIN

FACULTY POSITION TOXICOLOGY

The Division of Pharmacology and Toxicology in the College of Pharmacy has available a tenure-track position at the level of **Associate** or **Full Professor** with anticipated start date of Fall 2006. The successful candidate will have an established, nationally recognized research program that complements our departmental strengths in deciphering the mechanisms of reproductive toxicants, endocrine disruption, cell death and survival signaling, mitochondrial energetics and neuropharmacology; although other areas of research will be considered. Qualifications include a Ph.D. or equivalent doctoral degree, evidence of independent research funding and a strong publication record. We are especially interested in individuals with training in pharmacology/toxicology as the individual filling this position will participate in the teaching of both Pharm.D. and Ph.D. students. A competitive startup package and generous laboratory space is available. The Division of Pharmacology and Toxicology is a joint participant with the MD Anderson Cancer Center in a NIEHS Environmental Center Grant (**http://sciencepark.mdanderson.org/cred/**) as well as the site of two NIH predoctoral training programs in toxicology and alcoholism. Details of the pharmacology and toxicology educational program, as well as affiliated academic programs and associated research centers, can be found at: **http://www.utexas.edu/pharmacy/divisions/pharmtox/**.

Interested individuals should submit a curriculum vitae, a statement of their research plans, and the names of four references to: **Dr. John H. Richburg, The University of Texas at Austin, PHAR-Pharmacology, 1 University Station A1915, Austin, TX 78712-0125**; **john_richburg@mail.utexas.edu**. Application Deadline: **September 30, 2005**, but applications will be reviewed until a suitable candidate is selected.

UT-Austin is EO/AAE. Qualified women and minorities are encouraged to apply. This is a security sensitive position requiring a criminal background check of the successful applicant.

PEW Latin American FELLOWS PROGRAM in the BIOMEDICAL S·C·I·E·N·C·E·S

The Pew Latin American Fellows Program in the Biomedical Sciences provides support for young scientists from Latin America for post-doctoral training in the United States.

The sixteenth class of Fellows will be selected in 2006. An award of $50,000 will be provided as a salary stipend for the fellow during the period of training (2 years) and will be administered by the sponsoring U.S. institution. The sponsoring institution is required to supplement the salary stipend with at least $5,000 a year and to provide full medical benefits for the fellow. Following the two year fellowship, the Program will issue an additional $35,000 award to the sponsoring institution to purchase equipment and supplies for the fellow to establish a laboratory in his or her home country.

Applicants must have held a Ph.D. and/or M.D. degree, or equivalent, for no more than five years as of July 1, 2006. Applicants who received their degree from schools in the U.S., Canada or Europe will not be accepted. Applicants may not have had previous post-doctoral training outside of Latin America, nor may they have begun a post-doctoral position in the U.S. prior to July 1, 2005. Applicants are not required to have a commitment of a position and laboratory space after the fellowship. However, applicants must submit a written statement of intent to return to Latin America. Fellows must accept a position and have confirmed laboratory space in Latin America by the end of the fellowship period in order to obtain the $35,000 portion of the award.

Fellows will be selected on the basis of their promise as outstanding investigators, as well as the scientific merit of their research proposal, their record of training and how well their interests coincide with the laboratory of their sponsor in the United States. If potential applicants need assistance with the identification of an appropriate sponsoring laboratory in the United States, they may contact the Program Office before August 1, 2005. The program will accept applications from Mexico, Central and South America. Applications may be obtained from the Regional Committee contact listed here for each country or from our website at: **www.pewlatinfellows.com**

The application deadline is September 30, 2005. Winners will be notified in April 2006 and the fellowship should begin no later than August 2006.

APPLICATION DEADLINE IS SEPTEMBER 30, 2005

ARGENTINA
Ana Belén Elgoyhen, Chair
Instituto de Investigaciones en Ingeniería Genética y Biología Molecular
Phone: (5411)(4) 783-2871
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MEXICO
Mario Zurita, Chair
Universidad Nacional Autónoma de México
Instituto de Biotecnología
Phone: (52)(555) 622-7659
Fax: (52)(777) 317-2388
E-mail: marioz@ibt.unam.mx

All Other Countries
Silvia Montano de Jiménez
The Pew Latin American Fellows Program
3333 California Street, Suite 410
San Francisco, CA 94118
Phone: (415) 476-5116
Fax: (415) 502-4992
E-mail: montano@thecenter.ucsf.edu

Senior Postdoctoral Fellow/Scientist I

***ChemoCentryx** is a focused pharmaceutical company that creates first-in-class, orally available medicines for autoimmune diseases, inflammatory disorders and oncology. We are seeking a Senior Postdoctoral Fellow or Scientist I-level investigator to join our Drug Discovery team.*

The successful applicant will investigate the roles of chemokines and chemokine receptors in inflammation and cancer, and evaluate orally active small molecule antagonists as potential therapeutics. The work will involve establishing a variety of inflammatory disease models to access the efficacy of the company's drug candidates, and to elucidate the mechanisms of drug actions. Extensive experience in disease models and expertise in phenotypic and functional characterizations of immune cells are essential. A Ph.D. in immunology or a related discipline and 2+ years of postdoctoral training are required. In-depth knowledge of immunology and the immune system's roles in diseases preferred.

Please reference Job Code **CCX-410-SCI** and email your resume & cover letter to: **hiring@chemocentryx.com** or FAX to: **(650) 210-2910**. No phone calls please. *EOE*

ChemoCentryx

www.chemocentryx.com

POPULATION BIOLOGY and COMMUNITY ECOLOGY FACULTY POST-DOCTORAL FELLOWSHIPS in POPULATION BIOLOGY UNIVERSITY of NEBRASKA

The **School of Biological Sciences of the University of Nebraska** invites applications for two tenure-track faculty positions and two postdoctoral fellowships. One faculty position will be in the area of Community Ecology. The second position will be in the area of Population Biology, supported by University of Nebraska Program of Excellence funding to the School of Biological Sciences to strengthen research and teaching, in Population Biology, through faculty hires and a postdoctoral fellowship program. The positions are open at the Assistant or Associate Professor level. Candidates will be expected to develop (or to have already developed) a nationally recognized research program in Community Ecology or Population Biology and to teach undergraduate courses in biological diversity and/or ecology and evolution as well as graduate courses in their areas of expertise.

Review of applications will begin on **August 15, 2005**, with an expected start date of Fall, 2006. A Ph.D. in the life sciences is required and post-doctoral experience is preferred. To apply, indicate which position you are seeking, send a CV, representative publications, statements of research and teaching interests, and arrange for three letters of reference to be sent to: **Alan C. Kamil, School of Biological Sciences, University of Nebraska-Lincoln, 348 Manter Hall, Lincoln, NE 68588-0118**. These positions will remain open until suitable candidates are selected. Email address: **biologysearch@unl.edu**.

The **University of Nebraska Program of Excellence in Population Biology** announces two two year Postdoctoral fellowships in Population Biology. Candidates will develop a research project with a faculty member associated with the Program. Program details are available at (**http://popbio.unl.edu**). These positions will provide recent graduates the opportunity for independent research associated with a faculty sponsor (in Biological Sciences, Mathematics, Natural Resources, or Entomology). The Program of Excellence in Population Biology is an integrative, cross-disciplinary program and fellows will, therefore be expected to teach a cross-disciplinary graduate seminar each year.

Review of applications will begin **August 1, 2005**. A Ph.D. and expertise in any aspect of population biology is required. To apply, send a CV, a 5-page research proposal, and description of potential graduate seminars and arrange for three letters of reference, one of which must be from the proposed faculty sponsor, to the **Population Biology Post-doctoral Fellowship Selection Committee, School of Biological Sciences, University of Nebraska-Lincoln, 348 Manter Hall, Lincoln, NE 68588-0118**. Email address: biologysearch@unl.edu Program details are available at (**http://popbio.unl.edu**). Fellowship positions will remain open until suitable candidates are selected.

UNL is committed to a pluralistic campus community through Affirmative Action and Equal Opportunity, and is responsive to the needs of dual career couples. We assure responsible accommodation under the Americans with Disabilities Act. For further information contact Alan C. Kamil at 402-472-6676 for assistance.

ScienceCareers.org

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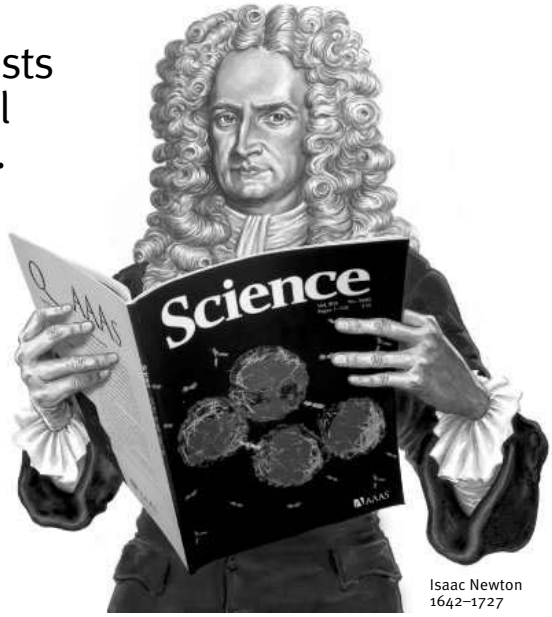

Isaac Newton
1642–1727

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Phone: 202-326-6534
E-mail: bdwyer@aaas.org

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THE HONG KONG POLYTECHNIC UNIVERSITY
香港理工大學

DEPARTMENT OF APPLIED BIOLOGY AND CHEMICAL TECHNOLOGY

Professor/Associate Professor/Assistant Professor

The Department of Applied Biology and Chemical Technology is a multi-disciplinary department in The Hong Kong Polytechnic University with diversified specialties in biology, chemistry, biochemistry, biotechnology, chemical engineering and food science. The departmental focus is on drug discovery and development with particular emphasis on herbal medicine.

The appointee will be required to (a) conduct lectures, practical sessions and tutorials at taught master, undergraduate and higher diploma levels in the broad fields of Pharmacology/Biotechnology, and especially in two or more of the following areas: (i) Molecular Pharmacology, (ii) Immunology and (iii) Human Physiology; (b) supervise postgraduate students at the MPhil and PhD levels; (c) establish a vigorous and externally funded research programme in line with the departmental focus which is on drug discovery and development with particular emphasis on herbal medicine; and (d) contribute to departmental and programme administration as well as curriculum development. Those appointed at Professor/Associate Professor level will be expected to take a leading role in the State Key Laboratory of Chinese Medicine and Molecular Pharmacology recently being established in Shenzhen by the University under the auspices of the Ministry of Science and Technology of The People's Republic of China.

Applicants should (a) have a PhD degree in related fields; (b) have at least ten, five and three years' post-qualification experience in drug discovery, development and application of modern technology in herbal drug development for the posts of Professor, Associate Professor and Assistant Professor respectively; and (c) be able to demonstrate evidence of effective classroom teaching skills. Applicants for the Professor post should also have a high level of academic achievement and standing in related fields, a substantial record and reputation as an accomplished teacher, and a distinguished and extensive track record in research and scholarly activities/high-level consultancy. Preference will be given to those with relevant industrial experience.

Remuneration

Salary offered will be commensurate with qualifications and experience. Initial appointment will be made on a fixed-term gratuity-bearing contract. Re-engagement thereafter is subject to mutual agreement. Remuneration package will be highly competitive. Applicants should state their current and expected salary in the application.

Application

Please submit application form via email to **hrstaff@polyu.edu.hk**; by fax at (852) 2764 3374; or by mail to **Human Resources Office, 13/F, Li Ka Shing Tower, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong.** Application forms can be obtained via the above channels or downloaded from **http://www.polyu.edu.hk/hro/job.htm**. **The closing date for application is Saturday, 20 August 2005.** Details of the University's Personal Information Collection Statement for recruitment can be found at **http://www.polyu.edu.hk/hro/jobpics.htm.**

Department of Molecular Medicine College of Veterinary Medicine - 04027 Cornell University

A Postdoctoral position is available for studies on cellular and molecular aspects of cancer metastasis. Studies will involve the molecular characterization of recently discovered cell-cell adhesion-activated signaling cascades and the role of these cascades in cancer cell survival and early metastatic growth. A PhD in molecular or cell biology, or biochemistry as well as a broad knowledge of the current biomedical literature are required.

Send curriculum vitae including names of 3 references to: **Dr. Bendicht U. Pauli, Cancer Biology Laboratories, Department of Molecular Medicine, Cornell University, College of Veterinary Medicine, Ithaca, NY 14853. E-mail: bup1@cornell.edu.**

Cornell University

Cornell University is an Affirmative Action/ Equal Opportunity, Employer and Educator

http://chronicle.com/jobs/profiles/2377.htm

GenomeCanada

VICE-PRESIDENT, RESEARCH

Genome Canada is seeking a Vice-President, Research who will be the *Voice of Genomics and Proteomics Research in Canada*.

Responsibilities include:

- Shape Canadian genomics and proteomics research into world leadership in key areas
- Ensure Canada's capabilities, competitiveness and exciting potential in this field of research
- Support Canada's most promising investigators
- Nurture relationships with Canadian and international stakeholder groups and Genome Centres across Canada

The candidate will be joining an innovative team in Ottawa, Canada, working in a rapidly evolving environment. You have the following qualities, experience and education:

- An advanced University degree and a respected history of discoveries, publications, awards, ideally focused on genomics/proteomics
- Clear academic recognition, with a history of peer reviewed support from national research funding agencies
- A broad personal and professional network of Canadian and international relationships among leaders and executives in diverse academic, industry and government settings
- Knowledge and experience in the area of Intellectual Property and commercialization
- Bilingualism (English and French) would be an asset.

The successful candidate will enjoy a competitive salary based on experience.

Full details about this unique opportunity and about Genome Canada are available at www.genomecanada.ca/Careers

Please forward your resumé by July 8, 2005
By email or fax to:

Hélène Meilleur
hmeilleur@genomecanada.ca
Fax: (613) 751-4474

POSTDOCTORAL RESEARCHER

Join the Lawrence Livermore National Laboratory (LLNL), Physical Biosciences Institute (PBI) and perform cutting-edge biosciences research in a multidisciplinary setting. PBI seeks to develop and apply new technologies for biophysical measurements leading to systems-level models of biological processes. You will be an integral member of a group of researchers with expertise in single-molecule spectroscopy, nanofabrication, confocal and atomic-force microscopy, accelerator mass spectroscopy, single-cell siRNA gene knockouts and molecular and systems simulations. You will work closely with staff from LLNL's Biology, Engineering, Chemistry, Physics and Computation directorates, and with university faculty, including faculty in the Natural Sciences Division at the new UC Merced campus. This position requires a recent Ph.D. in life sciences, chemistry, engineering, physics, computer science or mathematics; experience in biological experiments or modeling and the ability to perform research independently.

LLNL offers a challenging environment and competitive salary and benefits package. To view and apply for this position, go to http://jobs.llnl.gov, and search by job number **004152**. Submit your resume, publication list, names and addresses of three references and a short description of research interests and the scientific and technical roles that you envision having in our research team. LLNL is operated by the University of California for the Department of Energy's National Nuclear Security Administration. We are proud to be an equal opportunity employer with a commitment to workforce diversity.

University of California
Lawrence Livermore
NATIONAL LABORATORY

http://jobs.llnl.gov

MSSM
MOUNT SINAI SCHOOL OF MEDICINE

The Rosenstiel Department of Pharmacology and Biological Chemistry and Immunobiology Center, Mount Sinai School of Medicine seeks candidates for tenure-track faculty positions in Systems Biology at the Asst. or Assoc. Professor level to develop excellent research programs using experimental and theoretical approaches to understand the dynamics of complex physiological processes. Inflammation is a preferred focus, but other research areas will be considered. A translational approach related to human diseases will be encouraged.

Faculty Positions

Candidates must have an M.D. or Ph.D degree and postdoctoral training. We welcome applications from candidates with training in biomedical, physical, mathematical and engineering sciences and want to attract women and under represented minorities. Send CV, three publications, description of the proposed research program and names/addresses of three references to: **Systems Biology Search Committee, Mrs. Renny Satz-Grecco, Administrator, PBC Dept, Box 1215, Mount Sinai School of Medicine, One Gustave L. Levy Place, New York, NY 10029.** Electronic applications as PDF files may be sent to **renny.satz-grecco@mssm.edu**. EOE

DIRECTOR
STEM CELL RESEARCH
INSTITUTE OF MOLECULAR MEDICINE
UNIVERSITY OF CALIFORNIA, SAN DIEGO
SCHOOL OF MEDICINE

The University of California, San Diego School of Medicine seeks an established scientist with expertise in developmental biology to lead the program in stem cell research and regenerative medicine.

This position will report to the Director, College of Integrative Life Sciences/Dean for Translational Medicine, and will be responsible for the overall management, academic planning, resource allocation and program development and implementation of the Stem Cell Research Program. This individual should have a clear record of original research contributions and an active research program. The University and the School of Medicine have a strong commitment to advancing research in human stem cell biology commensurate with the commitment of the people of California. Appointment will be at the tenured full professor level with space and resources necessary to build an outstanding research program. Requirements include administrative experience in a complex research university or comparable setting. Other qualifications are experience in education and research, and significant academic experience and accomplishment, including teaching, research, public service and leadership. Salary is negotiable and commensurate with qualifications and experience.

Candidates should submit their curriculum vitae and bibliography, a summary of their research accomplishments, and the names and addresses of three (3) referees to: **Dr. Gordon Gill, Chair, Stem Cell Research Director Search Committee, c/o Lourdes C. Felix, 414 Pepper Canyon Hall, 9500 Gilman Drive, La Jolla, CA 92093-0602.** To ensure full consideration, letters of application should be received by **August 15, 2005**.

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Science
AAAS

Life Science Editor for *Science*

Join the dynamic team at *Science* as a full time associate editor for the biological sciences in our Washington, DC, USA or Cambridge, UK office. We are looking for a life scientist with broad interests, a lively curiosity, and experience in cutting-edge research in one or more of the following fields: evolutionary genomics, evo-devo, microbial ecology, biological chemistry, systems biology, bioinformatics, computational or quantitative biology, and neuroscience. Responsibilities include managing the review, selection, and editing of manuscripts, soliciting reviews and special issues, and fostering contacts and communication with the scientific community. Editors are expected to travel to scientific meetings. A Ph.D., postdoctoral experience, and multiple publications are required. Previous editorial experience is not necessary.

For consideration, send a resume and cover letter, along with salary requirements, to:

AAAS
Human Resources Department
Suite #101
1200 New York Avenue
Washington, DC 20005

Applications can also be sent by e-mail to **hrtemp@aaas.org** or Fax to **202-682-1630**. Visit us at: **www.aaas.org**.

Nonsmoking work environment. EOE.

SCHOOL OF MEDICINE
VANDERBILT UNIVERSITY

Faculty Position in Drug Disposition and Metabolism

The Division of Clinical Pharmacology at Vanderbilt University School of Medicine is recruiting a tenured or tenure-track faculty member in the field of **Drug Disposition and Metabolism**. Areas such as drug pharmacokinetics and pharmacodynamics, drug transporters/receptors, and mechanisms of drug metabolism are of high interest. Vanderbilt has a superb environment related to Pharmacology and Clinical Pharmacology and possesses outstanding cores to support this research.

Faculty Position in Lipid Mediator/Free Radical Pharmacology

The Division of Clinical Pharmacology at Vanderbilt University School of Medicine is recruiting a tenured or tenure-track faculty member in the field of **Lipid Mediator/Free Radical Pharmacology**. Areas such as eicosanoid biology/pharmacology, nitric oxide/free radical biology and biochemistry, and mechanisms of lipid mediator signaling are of high interest. Vanderbilt has a superb environment related to the pharmacology and clinical pharmacology of lipid mediators/free radicals and possesses outstanding cores to support this research.

Candidates with an M.D. and/or Ph.D. should send a curriculum vitae, statement of research interests, and at least three supporting letters from mentors or colleagues to: **Dr. Jason Morrow, Division of Clinical Pharmacology, Departments of Medicine and Pharmacology, 532 RRB, Vanderbilt University School of Medicine, Nashville TN 37232-6602; Tel. 615-322-4785; Fax 615-322-3669; email jason.morrow@vanderbilt.edu.**

Vanderbilt University School of Medicine is an Equal Opportunity/ Affirmative Action Employer.

MIT MEDIA LAB

Director
Media Laboratory / Media Arts and Sciences Program

The Massachusetts Institute of Technology invites nominations and expressions of interest for the position of Director of the Media Laboratory and Head of the Media Arts and Sciences Academic Program in the School of Architecture and Planning.

The Media Lab houses one of the world's leading research and academic programs, fostering the invention of new technologies and concepts in a multidisciplinary approach to human augmentation. It functions as an independently funded research organization situated within MIT, and grants degrees through its Media Arts and Sciences academic program.

The candidate must be a dynamic and visionary leader of internationally recognized accomplishment who is capable of inspiring and leading closely intertwined research and academic programs. This candidate must possess exceptional communication skills that are equally effective in intellectual and commercial environments. Academic stature warranting the rank of Full Professor at MIT is also essential. The preferred individual will possess a combination of for-profit and non-profit experience, as well as the ability to demonstrate both strategic and operational dimensions.

Please forward nominations and expressions of interest in confidence to:
Tod Machover, Chair, Search Committee
The Media Laboratory
Massachusetts Institute of Technology
20 Ames Street, E15-401
Cambridge, MA 02139
617-253-0617; hr@media.mit.edu

William Holodnak and Nancy Martin at J. Robert Scott, executive search consultants and a Fidelity Investments company, are assisting the MIT Media Lab, medialab@j-robert-scott.com.

An Equal Opportunity/Affirmative Action Employer

Bioscience Openings

Lawrence Livermore National Laboratory is seeking both senior and junior-level researchers, to establish a multi-disciplinary team of scientists focused on the functional and evolutionary systematics of microbial communities. The focus of this team will embrace the following three goals:

- environmental microbial metabolism: to understand how physical chemical forces shape the metabolic activity and organization of naturally occurring communities
- microbial regulatory architecture and dynamics: from genomic sequence to regulatory networks to systems level regulatory behavior
- predictive microbial genomics: toward developing the ability to predict a microbe's behavior and lifestyle directly from its genome, and by extension its impact and role within a community

Of particular interest is understanding whether useful predictions can be obtained that relate net metabolic impact to the properties of an environment. Combined experimental and theoretical/modeling approaches, augmented by technology development efforts, will be deployed to address this problem.

PhD-level candidates are sought with expertise in, though not limited to, one or more of the following areas:

1. experimental thermodynamic, biochemical, and physical-chemical analysis of microbial metabolism, the energy metabolism of communities in particular
2. instrumental approaches to metabolite and metabolic flux profiling, as well as community-scale proteomics
3. computational and/or experimental approaches to characterize protein complexes and biochemical pathway function
4. flux-balance, energy-balance, and other systems biology approaches to large-scale metabolic network inference, modeling and analysis
5. meta-genomic sequence analysis in the study of natural microbial communities
6. microbial comparative and evolutionary genomic analysis and genotype-to-phenotype mapping
7. the evolution of metabolic pathways and syntrophy relationships

We are seeking both a leader to head the new effort and additional scientific staff, including postdocs, who can contribute to it. Applicants should have an established and relevant research track record, be interested in pursuing the problem posed, and be motivated to work in a team context.

This new effort will be able to draw on a wide range of existing LLNL resources and expertise in areas such as high throughput sequencing, microbial genomics, proteomics, bioinformatics, world-class computational facilities, as well as a longstanding history of interdisciplinary research.

#003791 – DIVISION LEADER to head the new Microbial Systems Division
#003789 – multiple MICROBIOLOGIST positions
#003891 – multiple POSTDOCTORAL positions to investigate molecular mechanisms related to microbial adaptation to environmental conditions
#004065 & 004067 – two POSTDOCTORAL positions to develop novel computational tools for comparative genomics across a wide range of microbial species

LLNL offers a challenging environment and competitive salary and benefits package. To view and apply for this position, go to **http://jobs.llnl.gov**, and search by job number above. When applying and prompted please mention where you saw this ad. LLNL is operated by the University of California for the Department of Energy. We are proud to be an equal opportunity employer with a commitment to workforce diversity.

University of California
Lawrence Livermore
NATIONAL LABORATORY

http://jobs.llnl.gov

POSITIONS OPEN

NIST
National Institute of Standards and Technology

CHIEF, PHYSICAL AND CHEMICAL PROPERTIES DIVISION
National Institute of Standards and Technology

Applications are invited for candidates to lead the Physical and Chemical Properties Division at the National Institute of Standards and Technology (NIST) in Gaithersburg, Maryland, or Boulder, Colorado. The Division is the nation's reference laboratory for measurements, standards, data, and models related to (a) the properties of gases, liquids, and solids (thermophysical properties, thermochemical properties, interfacial properties), (b) rates and mechanisms of chemical reactions, and (c) fluid-based physical processes and systems. The Division, through its programs, supports NIST's mission to develop and promote measurement, standards, and technology to enhance productivity, facilitate trade, and improve the quality of life. The Division's research is carried out in six groups: (1) chemical reference data and (2) computational chemistry groups (located in Gaithersburg, Maryland); and (3) Thermodynamics Research Center (TRC), (4) experimental properties of fluids, (5) theory and modeling of fluids, and (5) Cryogenic Technologies Groups (located in Boulder, Colorado). Please refer to the Physical and Chemical Properties Division **website: http://www.boulder.nist.gov/div838/** for more information.

The Chief provides strategic vision and program direction for approximately 70 staff members including approximately 60 Ph.D. scientists with backgrounds in the physical sciences and engineering. Current projects focus on thermophysics and thermochemistry with major outputs in the form of technical reports, Standard Reference Data, Internet-based databases, calibrations, and Standard Reference Materials. The Division enjoys extensive collaborations with other federal governmental agencies and industry.

At NIST, Division Chiefs manage resources, conceive new programs, and develop complementary implementation strategies. Applicants must have demonstrated experience in leading and managing research, and a record of sustained scholarly accomplishments. Ideal candidates will have training and experience with the physical sciences and/or engineering. A Ph.D. in the physical sciences or engineering is desirable.

The base salary ranges from $103,947 to $140,300, depending on qualifications. *U.S. citizenship is required*. Applicants should send a resume and three references (names and contact information only) by post or e-mail to: **Mr. Neil Alderoty, Senior Management Advisor, Chemical Science and Technology Laboratory, National Institute of Standards and Technology, 100 Bureau Drive, M.S. 8300, Gaithersburg, MD 20899** or **e-mail: neil.alderoty@nist.gov.** Applications must be postmarked by July 31, 2005, and received no later than August 5, 2005.

At **website: http://www.usajobs.opm.gov,** see NIST-2005-ASF-BO or NIST-2005-ASF for physicist, chemist, or general engineer. *Department of Commerce is an Equal Opportunity Employer.*

The Department of Microbiology, Immunology, and Parasitology at Louisiana State University Health Sciences Center (LSUHSC) in New Orleans is seeking a researcher for a tenure-track position at the **ASSISTANT/ASSOCIATE PROFESSOR** level who has demonstrated excellence in the general area of tumor immunology. Research with potential clinical translation is encouraged. This position will be supported jointly with the Stanley S. Scott Cancer Center. External National Cancer Institute grants are required at the Associate Professor level. Please send curriculum vitae and the names of three references to: **Ronald Luftig, Chair of the Search Committee, Microbiology, Immunology, and Parasitology, 1901 Perdido Street, Box P6-1, New Orleans, LA 70112-1393.** *LSUHSC is an Equal Employment Opportunity/Affirmative Action Employer.*

POSITIONS OPEN

University of Massachusetts
UMASS Medical School

FACULTY POSITIONS
Department of Neurobiology
University of Massachusetts Medical School

The Department of Neurobiology, established as part of the unprecedented research expansion at the University of Massachusetts Medical School, has recently hired several outstanding faculty. We now solicit applications for additional tenure-track positions. The new Department augments an already existing interdisciplinary Program in Neuroscience. The laboratories for the Department are housed on one floor of a new state-of-the-art, 340,000 square-foot research building.

The Department seeks individuals of outstanding potential who are investigating fundamental mechanisms of brain function. Specific areas of emphasis include, but are not limited to, molecular, cellular, and developmental neurobiology and behavior. Candidates using vertebrate, *C. elegans*, or *Drosophila* model systems are welcome to apply. The positions are highly competitive with regard to startup funds, laboratory space, and salary. Rank will be commensurate with ability and experience.

Applicants should send curriculum vitae, statement of research interests, and names and addresses of three references to:

Dr. Vivian Budnik
Chair of Faculty Search Committee
Professor of Neurobiology
University of Massachusetts Medical School
364 Plantation Street
Worcester, MA 01605-2324

Visit neurobiology at **website: http://www.umassmed.edu/neurobiology/.**
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TROPICAL ECOLOGIST/ RESEARCH FORESTER

The Institute of Pacific Islands Forestry, Pacific Southwest Research Station, U.S. Department of Agriculture Forest Service, is seeking a Tropical Forest Ecologist to conduct research on native forest restoration and sustainability in Pacific Island landscapes. The Institute provides research and outreach to restore, preserve, and sustain forests of the Tropical Pacific (**website: http://www.fs.fed.us/psw/programs/ipif**).

The position will be located at the Institute's new Research Center on the campus of the University of Hawaii-Hilo. This is a full-time, permanent position with full health, retirement, and vacation benefits. The salary level will be at the GS-12, 13, or 14 grade depending on qualifications ($54,221 to $99,053 plus 16.5 percent cost-of-living adjustment). The successful candidate at the GS-13 or GS-14 level will lead the Restoration of Forest Ecosystems Research team. *Applicants must be U.S. citizens.* A full announcement (PSW-Demo-332-05 and PSW Demo 333-05) is available at **website: http://www.usajobs.gov.** Questions regarding this position should be directed to: **Dr. Julie S. Denslow** at **telephone: 808-933-8121** or **e-mail: jdenslow@fs.fed.us.**

POSTDOCTORAL POSITIONS
Developmental Functions of Rho Family GTPases

Two Postdoctoral positions are available for one year to study the signaling pathways mediated by Rho guanosine triphosphates. Candidates with experience working with mice and with molecular and cellular biology techniques are encouraged to send their curriculum vitae, statement of research interests, and the names of three references to: **Dr. Audrey Minden, Laboratory for Cancer Research, School of Pharmacy, Rutgers, The State University of New Jersey, 164 Frelinghuysen Road, Piscataway, NJ 08854-8020. Fax: 732-445-0687; e-mail: bachorik@rci.rutgers.edu.**

POSITIONS OPEN

School of Medicine
TEMPLE UNIVERSITY

ANGIOGENESIS RESEARCHER

Temple University School of Medicine is currently seeking a Researcher (Ph.D. or M.D.) at the **ASSISTANT** or **ASSOCIATE PROFESSOR** level who is trained in cell or molecular biology (preferably with a history of grant funding) and whose research interests lie in the area of vascular biology as related to cancer and its treatment. Areas of interest would be factors which control proliferation of the vascular endothelium, specified markers for the vascular endothelium, the role of vascular endothelium in controlling tumor cell growth, and the use of kininogen derivatives as adjuncts to cancer therapy, etc.

DNA REPAIR RESEARCHER

We wish to recruit a Researcher (Ph.D. or M.D.) at the **ASSISTANT** or **ASSOCIATE PROFESSOR** level who is trained in cell/molecular biology or molecular pathology/genetics (preferably with a history of grant funding) and whose research interests lie in DNA repair/recombination genes that are involved in drug resistance, breast, and/or ovarian carcinogenesis, etc.

Please send curriculum vitae and bibliography to: **Henry Simpkins, M.D., Ph.D., Professor and Chairperson, Department of Pathology and Laboratory Medicine** or **Robert Coleman, M.D., Professor and Director, Sol Sherry Thrombosis Research Center, Temple University School of Medicine, 3401 North Broad Street, Philadelphia, PA 19140.** *Temple University is an Equal Opportunity/Affirmative Action Employer and strongly encourages applications from women and minorities.*

MICROBIOLOGIST

California State University Fullerton, Department of Biological Science is seeking applicants for a full-time tenure-track position at the **ASSISTANT PROFESSOR** level with expertise in microbiology, to begin August 2006. Applicants must have a Ph.D. and postdoctoral research experience. The successful candidate will be expected to develop an active, externally funded research program involving undergraduate and Master's level students and must be committed to excellence in teaching at both levels. The person selected will join the Cell and Developmental Biology Concentration Group and The Center for Applied Biotechnology Studies, and will be expected to teach general microbiology, upper-division/Master's level courses in his or her area of specialization, and contribute to our inquiry-based, lower-division core course in cell biology. Send: (1) curriculum vitae (including a history of grant support), (2) a statement of research plans, (3) three related publications, (4) a three-part statement on teaching including (a) philosophy with description of pedagogical approaches, (b) experience, (c) preferences for upper-division elective courses, and (5) three letters of recommendation to:

Chair, Microbiology Search
Department of Biological Science
California State University
Fullerton, P.O. Box 6850
Fullerton, CA 92834-6850

Review of applications will begin September 12, 2005, and continue until a suitable candidate is appointed. **Website: http://biology.fullerton.edu.** *Women and minority candidates are particularly encouraged to apply. Affirmative Action/Equal Opportunity Employer/ ADA Employer.*

POSTDOCTORAL POSITIONS are available to study cytokine signal transduction, transcription factors, hypoxia, and heart development. Candidates with experience in molecular biology and/or knock-out/transgenic animals should send their curriculum vitae to **e-mail: yxy36@cwru.edu.** Contact:

Dr. Yu-Chung Yang
Department of Pharmacology
Case Western Reserve
University School of Medicine

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E-mail: jhannaford@sciencemag.jp

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We know science AAAS

POSITIONS OPEN

Los Alamos NATIONAL LABORATORY

A **POSTDOCTORAL POSITION** is available immediately for performing computer simulations of the ribosome and basic research related to the ribosome. Strong programming and parallel computing skills are required. Experience in message-passing interface and rational drug design are desired. Candidates should enjoy a highly competitive atmosphere and be interested in performing landmark calculations. Candidates must have a Ph.D. in physics, computer science, chemistry, biochemistry, or related field. Salary starts at $60,000 with benefits available. The postdoctoral fellow will have access to large allocations of computer time on the LANL "q-machine" and newer facilities (starting October 2005). Applicants must send curriculum vitae, statement of research interests, and three references to: **Kevin Y. Sanbonmatsu (e-mail: kys@lanl.gov), MS K710, Los Alamos National Laboratory, Los Alamos, NM 87545.**

FACULTY POSITION
ASSISTANT PROFESSOR
Department of Pharmacology

Applications are invited for a full-time, tenure-track Assistant Professor position in the Department of Pharmacology in the School of Pharmacy, University of Mississippi (Oxford campus). We seek an individual who will establish and maintain a research program in pharmacology, teach in the core curriculum of the professional and graduate program, and participate in service-related activities. Applicants must have a Ph.D. degree in pharmacology, toxicology, or a closely related field, with postdoctoral experience and a strong record of research accomplishments. High priority areas of research emphasis include molecular pharmacology, neuropharmacology, cardiovascular/autonomic pharmacology, endocrine pharmacology, and environmental toxicology. Exceptional candidates in other research areas will also be considered. Ample opportunities exist for collaborative research programs with members of the National Center for Natural Products Research and the Research Institute of Pharmaceutical Sciences. Visit our **website: http://www.olemiss.edu/depts/pharmacology** for additional information about the research and training programs of faculty in the Department of Pharmacology at the School of Pharmacy.

Applications must be submitted online at **website: https://jobs.olemiss.edu.** Applicants shall provide a letter outlining their research interests, teaching experience and qualifications, complete curriculum vitae, and names, addresses, telephone numbers, and e-mails of three references. Review of applicants will begin immediately and continue until the position is filled. The anticipated starting date is January 2006.

The University of Mississippi is an Equal Employment Opportunity/Affirmative Action/Title VI/Title IX/Section 504/ADA/ADEA Employer.

RESEARCH FACULTY POSITION AVAILABLE: The Department of Neurology at Columbia University is seeking an **ASSOCIATE RESEARCH SCIENTIST.** The position available is in a laboratory studying cell biology of neurodegeneration associated with Parkinson's disease and related disorders. We are particularly interested in the exploration of mitochondrial function in models of Parkinson's disease. A variety of knockout and transgenic systems are available to the candidate. The preferred candidate should hold an M.D. or Ph.D. and have experience in cell or molecular biology, in a variety of assays used to study mitochondrial function coupled with interest in mechanisms involved in neurodegeneration. Application, including curriculum vitae and references and a description of research interests and experience, should be sent to: **Dr. Serge Przedborski, Professor, Columbia University, Department of Neurology, 650 W. 168th Street, BB-309, New York, NY 10032.** *Columbia University is an Affirmative Action/Equal Opportunity Employer.*

POSITIONS OPEN

STERIS

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At our world headquarters, in Mentor, Ohio, we seek well-rounded, creative individuals to fill the following positions: **SENIOR SCIENTIST**–This person will be conducting studies in a regulated environment, within both chemical and microbiological disciplines, contributing to sterilization technology product development. Studies may include, but will not be restricted to: (1) adjustments to existing or development of new chemical formulation needed to generate required level of sterilant and (2) working to develop indicator organisms or chemical integrators to demonstrate exposure to a liquid or vapor sterilant at the proper concentration for the required time. The successful candidate will have a Ph.D. and three to five years of experience. Candidates must have proven product development experience, as well as familiarity with working in U.S. Food and Drug Administration or U.S. Environmental Protection Agency compliance environments.

SCIENTIST–For this position, a background in physical or materials chemistry would be valuable as well as experience with vacuum or gas delivery systems. The successful candidate will have a B.S. with five to ten years experience or a Ph.D. with three to five years of experience. Candidates must also have a proven record in product development and release.

To learn more and to apply online, please visit **website: http://www.steris.com.** Please note that only applications received via **website: http://www.steris.com** will be considered.

Equal Opportunity Employer.

Positions are immediately available in the Section of Digestive Diseases, West Virginia University (WVU). Candidates are invited to apply for two positions: **RESEARCH ASSISTANT PROFESSOR** and/or **RESEARCH SENIOR INSTRUCTOR.** The major areas of interest include regulation of intestinal electrolyte and nutrient transport in the normal and chronically inflamed intestine and determination of the intracellular pathways that mediate these alterations, and the molecular characterization of altered transport pathways. These are nontenure-track National Institutes of Health supported positions for a minimum of four years. The successful applicant should ideally have pre- and postdoctoral training and expertise in molecular biology. Alternatively candidates may have pre-doctoral background in physiology or cell biology with postdoctoral training and expertise in molecular biology. Expertise in cloning, promoter analysis, and phosphorylation is necessary. Expertise in transport physiology and intracellular signaling is preferred. Submit curriculum vitae and names/addresses of three references to:

David DiBartolomeo, Administrator
Digestive Diseases
P.O. Box 91614
Morgantown, WV 26506
E-mail: ddibartolomeo@hsc.wvu.edu
Telephone: 304-293-6743

WVU is an Equal Opportunity/Affirmative Action Employer. Women and Minorities are encouraged to apply.

POSITIONS OPEN

UW Medicine
SCHOOL OF MEDICINE

UNIVERSITY OF WASHINGTON SCHOOL OF MEDICINE

Howard Hughes Medical Institute (HHMI)-funded **POSTDOCTORAL POSITION** available in the Department of Genome Sciences, University of Washington, Seattle, Washington, to study rapidly evolving duplicated genes that lack orthologs in model organisms. We seek an individual with expertise in antibody/immunostaining techniques, tissue histology and imaging, mouse transgenics, and expression microarray technology to study novel protein products in the context of human and mouse transgenic tissues. Expertise in genomics and molecular evolution are also preferred. Applicants must have a Ph.D. in a field of molecular biology, be within two years of their Doctorate and have demonstrable expertise in the forementioned areas. The position is for three years. Starting salary will be up to $41,000 with HHMI benefits including allowable moving expenses to Seattle, Washington. Please send curriculum vitae and contact information of three references to: **Evan Eichler, Ph.D. (e-mail: eee@gs.washington.edu), Department of Genome Sciences, Box 357730, University of Washington, Seattle, WA 98195.**

ASSISTANT, ASSOCIATE, AND FULL PROFESSORS
Department of Physiology
University of Kentucky Medical Center

We seek three bright, motivated colleagues to join our tenured or tenure-track faculty at any rank. **Website: http://www.mc.uky.edu/physiology.** Biomedical scientists with a Ph.D., M.D., or equivalent degree and at least two years of postdoctoral research training are encouraged to apply. Applicants must possess expertise in modern scientific methods and be competitive for extramural funding at the national level. Preference will be given to individuals with innovative approaches that complement existing research programs in the areas of respiratory biology, neurodegenerative disease, spina l cord injury, sensory neuroscience, cardiovascular science, muscle biology, renal physiology, reproductive endocrinology, free radical biology, and aging. In addition to research, successful applicants will participate in the teaching mission of the Department and will be involved in our graduate-training program. We are committed to the success of our faculty and provide modern laboratory space, protected research time, and startup funds. We also provide a mentoring program for junior faculty. Interested individuals should forward their curriculum vitae, a one-page summary of research interests, and the names of three references to: **Ms. Bonnie Emerich, Search Committee Administrator, Department of Physiology, University of Kentucky Medical Center, 800 Rose Street, Room MS-508, Lexington, KY 40536-0298.** *The University of Kentucky strongly encourages applications from women, minorities, and people with disabilities and is an Affirmative Action/Equal Opportunity Employer.*

PSYCHOPHARMACOLOGY FACULTY SEARCH

The Department of Psychiatry and Behavioral Sciences at Stanford University School of Medicine is seeking two full-time faculty members in the area of psychopharmacology. One of these positions will be at **ASSISTANT, ASSOCIATE,** or **FULL PROFESSOR** level and based at the Veterans Affairs Palo Alto Health Care System; the second position will be at the **ASSISTANT PROFESSOR** level and based in the Stanford University Hospital and Clinics. Applicants must hold an M.D. or equivalent degree and board certification in general psychiatry. Applicants should forward curriculum vitae and the names and addresses of five references to: **K. Thomas, Faculty Affairs Administrator, 401 Quarry Road, Stanford, CA 94305-5717.** *Stanford University is an Equal Opportunity/Affirmative Action Employer.*

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Mr. Jensen has over 20 years of experience in human resource consulting and staffing for the biotechnology and pharmaceuticals industry.

Adviser Bill Lindstaedt
Director, UCSF Career Center
Mr. Lindstaedt has been providing career related advice to scientists and engineers for nearly 15 years, with a particular emphasis on working with graduate-level trainees in the life sciences.

Adviser Naledi Saul
Assistant Director, UCSF Career Center
Ms. Saul has 7 years of career counseling with 4 years focused on counseling graduate students and postdocs in the biomedical and health sciences. Her forte is working with scientists pursuing careers in the public health arena.

Adviser Jim Austin
Editor, Science's *Next Wave*
Dr. Austin has a Ph.D. in physics and worked in academia before coming on board to write about traditional and nontraditional career paths for scientists.

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CASE
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POSTDOCTORAL POSITIONS are available for recent graduates (Ph.D. and/or M.D./Ph.D.) who are interested in studying chromatin-remodeling in skeletal muscle cells and in cells expressing the adenovirus E1A protein. Example of recent publications: *Proc. Natl. Acad. Sci.* **100:**1735, 2003; *Mol. Cell* **12:**255, 2003. Applicants should forward their curriculum vitae and three names of references to: **Dr. M. L. (Nikki) Harter** at **e-mail: ml.nikki.harter@case.edu.** All qualified applicants are encouraged to apply; *however, in accordance with NIH requirements, U.S. citizens will be given first preference.*

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University of Massachusetts
UMASS Medical School

TENURE-TRACK FACULTY POSITION
Department of Cell Biology
University of Massachusetts Medical School
Worcester, Massachusetts

Applications are invited for a tenure-track faculty position at the **ASSISTANT PROFESSOR** level. Candidates of outstanding research potential are being sought to develop an extramurally funded program within the areas of developmental biology and genetics, particularly related to mammalian systems (mouse and human) from a cellular perspective and/or as linked to cancer. The position is highly competitive with regard to salary, startup funds, and new laboratory space.

The University of Massachusetts Medical School and Graduate School of Biomedical Sciences are undergoing rapid growth. Excellent core facilities, including genomics, proteomics, microscopy, digital imaging, and transgenic/knockout mice are provided. The Department has been ranked among the top cell biology research programs in the country. Send letter of application with curriculum vitae, statement of accomplishments and research plans, and the names and addresses of three references as a PDF file to: **Dr. Jane B. Lian, Search Committee Chair** or **Dr. Gary S. Stein, Department Chair** at **e-mail: cellbiosearch@umassmed.edu.** *An Equal Opportunity/Affirmative Action Employer.*

The Department of Medicine at the University of California San Francisco (UCSF) is recruiting physician-scientists engaged in translational research. Candidates must have an M.D. or M.D./Ph.D. degree and demonstrated potential to lead a first-rate and independent research program. Board certification in internal medicine is required. Appointments will be made at the **ASSISTANT/ASSOCIATE PROFESSOR** level in the In-Residence series, depending upon qualifications. The candidate will also become a member of the graduate program in biomedical sciences. Please send curriculum vitae to:

Joseph M. McCune, M.D., Ph.D.
Chair Search Committee
Gladstone Institute of Virology and Immunology
1650 Owens Street
San Francisco, CA 94158

UCSF is an Affirmative Action/Equal Opportunity Employer. The University undertakes affirmative action to assure equal employment opportunity for underutilized minorities and women, for persons with disabilities, and for Vietnam-era veterans and special disabled veterans.

INSTRUCTOR POSITION available in Department of Biochemistry, The University of Texas Southwestern Medical Center for crystallographic and functional studies of macromolecular catalytic machines. The applicant for this position should have a recent Ph.D. and postdoctoral experience with first-author publications in X-ray crystallography. Send curriculum vitae and contacts for three references to: **Dr. David T. Chuang** at **e-mail: david.chuang@utsouthwestern.edu.** *UT Southwestern is an Equal Opportunity/Affirmative Action Employer.*

POSITIONS OPEN

THE UNIVERSITY OF IOWA

POSTDOCTORAL POSITION

A Postdoctoral fellowship position is available in the laboratory of **Dr. Nicholas Zavazava** at the University of Iowa (see: *Nature Med.* **8:**171–178, 2002; *Blood* **99:**3286–3292, 2002; *Transplantation* **79:**1040–1044, 2005). Applicants must have a Ph.D. and should have experience in immunology, cell biology, or signaling. The laboratory currently focuses on human and murine embryonic stem cells. Applications, including curriculum vitae and bibliography, summary of past accomplishments, and names of three references should be sent to: **Nicholas Zavazava, M.D., Ph.D. Telephone: 319-384-6577; e-mail: nicholas-zavazava@uiowa.edu.**

The University of Iowa is an Equal Opportunity and Affirmative Action Employer. Women and minorities are strongly encouraged to apply.

ASSISTANT OR ASSOCIATE PROFESSOR

The Department of Surgery at the University of Pennsylvania's School of Medicine seeks candidates for an Assistant or Associate Professor position in the tenure track. Rank will be commensurate with experience. The successful applicant will have experience in the field of urology with a focus on pathology, cell/molecular biology, or biochemistry. Responsibilities include targeted research in urothelial biology, interstitial cystitis, and tissue and cell engineering to correct urologic disorders, as well as teaching of medical students and residents doing research rotations in the urology laboratories. Applicants must have an M.D. and/or Ph.D. degree.

This position will include teaching and research duties only, with no patient care responsibilities. The successful candidate will have demonstrated potential for establishing a vigorous independent research program in the cellular/molecular basis of diseases of the lower urinary tract. Preference will be given to candidates with experience in basic urological research.

Please submit curriculum vitae, a brief statement of research interests, and references to: **Dr. Alan Wein, Chief, Division of Urology, c/o Peter Atherton, University of Pennsylvania School of Medicine, 3400 Spruce Street, 4029 Maloney, Philadelphia, PA 19104-4283. E-mail: athertop@uphs.upenn.edu.** *The University of Pennsylvania is an Equal Opportunity/Affirmative Action Employer. Women and minority candidates are strongly encouraged to apply.*

ENDOTHELIAL/STEM CELL SCIENTIST

Startup biotechnology company in Research Triangle Park, North Carolina, focused on tissue engineering seeks a Ph.D.-level scientist with experience with endothelial cells and/or stem cells to develop and implement scientific projects. The successful applicant has creative scientific ideas, works well both independently and in teams, and has good oral and written communication skills. Send resume/curriculum vitae to **e-mail: humacyte@humacyte.com.**

Additional job postings not featured in this issue can be viewed online at **website: http://www.sciencecareers.org.** New jobs are added daily!

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Head, Cardiovascular Research University of Maryland School of Medicine

The Department of Medicine at the University of Maryland School of Medicine is recruiting a Director for Cardiovascular Research at the faculty rank of Associate Professor/Professor, tenure status dependent upon qualifications. The successful candidate for this senior leadership position within the Cardiology Division and the Maryland Heart Center will have a focus on laboratory based research in the areas of heart failure, vascular biology, or cellular electrophysiology, and have a proven track record for obtaining peer reviewed extramural funding. The position comes with an attractive package to foster the development and growth of a robust research program – resources for additional faculty recruitment are also available.

Those interested should provide a current resume and names of four references and forward to the attention of **Mandeep R. Mehra, MD (Search Committee Chair), Chief of Cardiology (email: mmehra@medicine.umaryland.edu) c/o JoAnn Gibbs, Academic Programs Office, Department of Medicine, Room N3E10, 22 South Greene Street, Baltimore, MD 21201**. Reference Position **#03-309-427**.

The UM,B encourages women and members of minority groups to apply and is an AA/EEO/ADA Employer.

The **National Institute of Allergy and Infectious Diseases (NIAID)**, a major research component of the NIH and the Department of Health and Human Services, is recruiting for one **Post-doctoral Fellow** or **Research Fellow**. The position will be available in the Respiratory Viruses Section of the Laboratory of Infectious Diseases. The research program focuses on molecular biology, pathogenesis, host immune response, reverse genetics and vaccine design for human respiratory syncytial virus and human metapneumovirus, and the use of paramyxoviruses as vectors for highly pathogenic agents (*J Virol* 2005, **79:**6588-97; 5353-62; 2004, **78:**13362-5; 2003, **77:**11201-11; *JID* 2005, **191:**1093-104; *Lancet* 2004, **363:**2122-7; *PNAS* 2004, **101:**9804-9).

The salary range for post-doctoral applicants is $38,500-56,900, depending on experience. Research Fellow applicants should have three years or more experience; the salary range is $40,974-72,990.

Applicants should send their curriculum vitae and names and addresses of three (3) references to:

Peter Collins
50 South Drive MSC 8007
Bethesda, MD 20892-8007

FAX: (301) 496-8312
email: pcollins@niaid.nih.gov

FELLOWSHIPS

POSTDOCTORAL FELLOWSHIPS

Applications are being accepted for the Philip Morris External Research Program Postdoctoral Fellowships. The Postdoctoral Fellowships program was established to support the development of scientists at the beginning of their careers in the following scientific areas: Exposure/Biomarkers/Dosimetry, Epidemiological Research, Clinical and Model Systems Research, and Tobacco Smoke and Smoking Behavior.

Qualified applicants must hold a recently conferred Ph.D. degree, M.D. degree, or equivalent degree at the time of the award. Candidates should have no more than four years of postdoctoral research experience and have demonstrated an ability to conduct independent research. This is an international program and there are no citizenship or residency requirements for the Postdoctoral Fellowships.

Sponsorship of the fellow by an established investigator is required. There are no further restrictions regarding the specific discipline of the sponsor or the department at which the research will be conducted. International sponsors and collaborations are encouraged.

Applications must be received by **October 1, 2005**. For more information on the Postdoctoral Fellowships Program, please call or write: **Research Management Group, Philip Morris External Research Program, Postdoctoral Fellowships, 1099 Winterson Road, Suite 280, Linthicum Heights, MD 21090-2216 USA; Telephone: 1-410-684-3782; Fax: 1-410-684-3729; Email: rmgroup2000@aol.com.**

AWARDS

Department of Veterans Affairs
Office of Research and Development
Biomedical Laboratory Research and Development Service

2004 William S. Middleton Award

Was presented to:
John C. Crabbe, Ph.D.
VA Medical Center, Portland, OR

The Middleton Award is the Biomedical Laboratory Research and Development Service's highest scientific honor, awarded annually to a senior VA investigator for accomplishments in areas of prime importance to the VA's research mission.

Dr. Crabbe was especially honored for his original and seminal contributions to the field of behavioral genetics, particularly as it relates to alcoholism. His research has had a significant impact on understanding the genetic bases and behavioral consequences of alcohol intake. His work with animal models has advanced the field of behavioral genetics, and it has important implications for showing the complexity of analogous traits or phenotypes underlying alcohol drinking behavior and alcoholism in humans.

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AWARDS

BODOSSAKI FOUNDATION

BODOSSAKI ARISTEIO PRIZE 2006

The Aristeio Bodossaki was instituted to give recognition to Greeks who have devoted their lives to science and who, by means of their exceptional, lifelong performance and significant achievements, have made a distinctive contribution towards furthering their field of science. The Aristeio Bodossaki which is accompanied by the sum of €150.000, is awarded every two years.

The Board of Trustees of the Bodossaki Foundation, at its meeting in May 2005, accepted the proposal of the International Aristeio Committee to award the Bodossaki Aristeio for the year 2006 in the field of Mathematics. The Committee recommended that the prize be shared by two outstanding, world-class mathematicians, Professor A. S. Fokas of Cambridge University, and a member of the Academy of Athens for his contributions to the solution of problems in Nonlinear Partial Differential Equations and in applications of these advances in many diverse scientific fields and Professor D. Christodoulou of ETH-Zürich for his rigorous contributions to the theory of General Relativity and Gravitation. Both have been awarded numerous prestigious international awards and their work has a big impact on researchers in their fields.

The Jacob P. Waletzky Memorial Award for Innovative Research in Drug Addiction and Alcoholism

The Society for Neuroscience is pleased to announce the Call for Nominations for the Jacob P. Waletzky Memorial Award. This prize will be awarded each year at the SfN annual meeting to a young scientist who has received an advanced degree of either a PhD or MD within the past fifteen years, and who has done research or plans to do research in the area of substance abuse and the brain and nervous system. The award is $25,000.

Nomination packages should include the following:

- An essay of not more than 500-words describing the future goals and direction of their planned research in the area of substance abuse and the brain and nervous system.
- Up to 3 letters of recommendation. Only one letter can be from the applicant's institution and only one can be from a current or former mentor (graduate student or postdoctoral advisor). The third letter should be from an individual who has not worked with the applicant. No individual may recommend more than one applicant.
- Supporting documents, such as a curriculum vitae and a list of all publications and abstracts authored or co-authored by the applicant.

A selection committee will review all applications and will select the awardee. Because of potential conflicts of interest issues, members of the Award Committee cannot serve as nominators nor should they write letters of support.

Nomination packages for The Jacob P. Waletzky Memorial Award should be submitted to: **B. Lawrance, Society for Neuroscience, 11 Dupont Circle, NW, Suite 500, Washington, DC 20036** or **beth@sfn.org.**

Deadline for Receipt of Nomination Packages:
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- The next deadline for receipt of applications is August 1, 2005. Full applications with all materials should be submitted directly to office listed below.
- Investigators must submit a 1-2 page *Letter of Intent* summarizing the proposed project at least 15 days before the deadline.
- Further information about this program can be found at: **http://dtp.nci.nih.gov**
- Inquiries can be made to the RAID Program Coordinator by telephone at 301-496-8720 or by e-mail at:

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Developmental Therapeutics Program
National Cancer Institute
6130 Executive Blvd., RM 8022
Rockville, MD 20852
Tel: 301-496-8720; Fax: 301-402-0831
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